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Research Article

Deltamethrin Resistance in Field Populations of *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) Collected from Paddy Rice Warehouses in Taiwan

Wen-Bin Feng[1], Chi-Yang Lee[1], and Me-Chi Yao[2,*]

Abstract

Feng, W. B., C. Y. Lee, and M. C. Yao. 2025. Deltamethrin resistance in field populations of *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) collected from paddy rice warehouses in Taiwan. J. Taiwan Agric. Res. 74(1):1–10.

The lesser grain borer, *Rhyzopertha dominica* (Fabricius) is one of the most common stored product pests found in the paddy rice warehouses in Taiwan. Both larvae and adults can bite through the husk of paddy rice, causing serious losses. Currently, 0.055% dustable powder of deltamethrin is the only insecticide applied in the paddy rice warehouses to control the lesser grain borer in Taiwan. Deltamethrin has been recommended to control the stored product pests for 21 years since 2002. The long-term use of insecticides may lead to the development of insect resistance. In this study, we collected 21 *R. dominica* populations from paddy rice warehouses and obtained the deltamethrin resistance ratio of *R. dominica* with the rice-mixing method. The highest resistance ratios to deltamethrin were found in Wufeng, Hemei, Lukang, Minxiong, Liujiao, Houbi, Wandan, Dongshan, Yilan and Sanxing with the resistance ratios exceeding 117.65-fold. Results showed that *R. dominica* populations have developed high resistance to deltamethrin after a decade-long use of deltamethrin in Taiwan. To solve effectively control the lesser grain borer, it is necessary to find the alternative insecticides for controlling the stored product pests in paddy rice warehouses in Taiwan.

Key words: Paddy rice warehouse, *Rhyzopertha dominica*, Deltamethrin, Lethal concentration 50, Resistance ratio.

INTRODUCTION

The lesser grain borer, *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae), one of the primary stored product pests, mainly damaging corn, rice, wheat, and other cereal-stored products. It is widely distributed worldwide, including in the United States, Europe, India, China, and Australia (Edde 2012), and it is the most serious pest in paddy rice warehouses in Taiwan (Yao *et al*. 2022). Adults lay eggs on the surface of paddy rice, and both larvae and adults can bite through the paddy rice husk to invade the interior of the rice, causing damage. Its damage often produces rice powder, which could cause the outbreak of secondary pests such as the rusty grain beetle (*Cryptolestes ferrugineus* Stephens), the saw-toothed grain beetle (*Oryzaephilus surinamensis* (Linnaeus)), and the foreign grain beetle (*Ahasverus advena* (Waltl)). The lesser grain borer often leads to huge losses of rice and downgrades food safety in Taiwan.

To control damages caused by *R. dominica* during paddy rice storage, 0.055% dustable

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powder (DP) of deltamethrin was mixed evenly with paddy rice at a ratio of 1 : 750 in Taiwan, called the rice-mixing method. The Agriculture and Food Agency purchases a certain amount of deltamethrin commodities and delivers them to the managers of paddy rice warehouses across Taiwan every year. Using 0.055% deltamethrin DP had been recommended for controlling the lesser grain borer since 2002 (Yao & Lo 2000). The long-term use of insecticides with a single mechanism of action increased the stored product pests' chances of developing insecticide resistance. In recent years, many managers of paddy rice warehouses have frequently reported that deltamethrin is ineffective in controlling stored product pests such as the lesser grain borer and the Angoumois grain moth (*Sitotroga cerealella* (Olivier)). Additionally, some managers of warehouses would apply deltamethrin DP to the surfaces of 60-kg storage bags or above ton storage bags rather than mixing it with paddy rice. Since deltamethrin is a contact-type insecticide, the control effect of applying it to the storage bag surface could be ineffective. These incorrect application methods of deltamethrin fail to achieve the expected control effectiveness, increase unnecessary costs, and raise the risk of resistance development in stored product pests to deltamethrin. *R. dominica* resistance to deltamethrin has been reported in many countries, including Taiwan (Chen & Chen 2013), Australia (Daglish & Nayak 2018), Brazil (Lorini & Galley 1999), and India (Ramesh Babu *et al*. 2017a, 2017b). Chen & Chen (2013) first revealed the presence of deltamethrin resistance in *R. dominica* with the highest resistance ratio (RR) of 63.8 in Nantou, Taiwan. After ten more years of using deltamethrin, the deltamethrin resistance in *R. dominica* populations may have increased even more.

In this study, *R. dominica* adults were collected from paddy rice warehouses across Taiwan, and the LC_{50} of deltamethrin of each strain was determined to understand the development of deltamethrin resistance in *R. dominica* populations.

MATERIALS AND METHODS

Sampling site and insect rearing

Adults of *R. dominica* were collected from 21 paddy rice warehouses in northern, central, southern, and eastern Taiwan (Fig. 1) between September 2022 and October 2023.

The collected adult beetles were reared in plastic containers (radius: 12 cm; height: 5 cm) with oatmeal as their food source. Additionally, a strain that had not been exposed to insecticides for at least 10 years in the laboratory was used as the most susceptible population in this study. All strains were maintained in the Laboratory of Integrated Pest Management, Applied Zoology Division, Taiwan Agricultural Research Institute, in environmental chambers with a temperature set at 27 ± 1℃ and relative humidity at 70 ± 5%, and 24-h darkness.

Insecticide

A technical-grade deltamethrin (99.4%; Dr. Ehrenstorfer GmbH, Augsburg, Germany) was used to examine the deltamethrin resistance of *R. dominica* strains collected from each warehouse. The deltamethrin was diluted with acetone to the respective concentrations for insecticide resistance bioassays.

Insecticide resistance bioassays

To simulate the actual application of deltamethrin DP (the recommended formulation in Taiwan) in paddy rice warehouses, the rice-mixing method was employed in this study (Yao & Lo 2000). Erlenmeyer flasks (radius: 2.5 cm; height: 9 cm) were prepared with 0.1 g of talcum powder, into which 1 mL of each concentration of deltamethrin was dripped onto the bottom of the flask. The mixture of talcum powder and deltamethrin solution was air-dried at 27 ± 1℃ and 70 ± 5% relative humidity for 3 h. The 10 g paddy rice (cultivar: 'Tainan No. 11') grains were poured into the flask, and mixed with the deltamethrin-treated talcum powder using a Vortex mixer (Scientific Industries, Inc., New York, USA). Thirty unsexed

Fig. 1. Locations where *Rhyzopertha dominica* adults were collected from paddy rice warehouses in Taiwan. ● denotes *R. dominica* populations with a high degree of deltamethrin resistance.

F_2-F_3 *R. dominica* adults (2-to-3 weeks old) were added into the flask to initiate the experiment. Each strain was treated with five to seven concentrations of deltamethrin, ranging from 0.01 mg mL^{-1} to 10 mg mL^{-1}, and the control was treated with acetone only. Each concentration was replicated three times. All treatments were maintained at 27 ± 1℃, 70 ± 5% relative humidity, and a 12 : 12 h photoperiod for 24 h. Mortality was observed after 24 h, and individuals with no response to touching were considered dead.

Statistical analysis

The concentration causing 50% *R. dominica* population mortality (LC_{50}), the 95% fiducial limits (FL) of LC_{50}, and the chi-squared test were obtained using Probit analysis in the SPSS program (Version 23). The resistance ratio of each strain was generated from the LC_{50} of each strain divided by the LC_{50} of the laboratory strain in this study. If the mortality was less than 50% at 10 mg mL^{-1} deltamethrin, the LC_{50} was stated as > 10 mg mL^{-1} while the resistance ratio was shown as > 117.65-fold.

The degrees of resistance ratio were classified as follows: low for RR ≤ 10, moderate for 10 < RR ≤ 40, high for 40 < RR ≤ 160, and extremely high for RR ≥ 160 (Hayashi 1983).

RESULTS

Among the 22 populations of *R. dominica* (Table 1), the Lab strain required the lowest concentration of 0.085 mg mL^{-1} to cause 50% population mortality. The highest deltamethrin resistance was found in several strains collected from Wufeng, Hemei, Lukang, Minxiong, Liujiao, Houbi, Wandan, Dongshan, Yilan, and Sanxing, all with the LC_{50} greater than 10 mg mL^{-1}. Five strains were determined from northern Taiwan, among which *R. dominica* populations of Tongluo were classified as moderate resistance to deltamethrin, with a resistance ratio of 10.31-fold. The strain from Yuanli was classified as high deltamethrin resistance, with a resistance ratio of 48.53-fold compared with

Table 1. Susceptibility, resistance ratio (RR), and degree of resistance of *Rhyzopertha dominica* populations to deltamethrin in paddy rice warehouses in Taiwan.

Strain	N[z]	LC_{50} (95% FL[y]) mg mL^{-1}	Slope	χ^2	*df*	RR[x]	Degree of resistance[w]
Laboratory	450	0.085 (0.044–0.139)	1.56	38.66	13	1.00	Low
Northern							
Yangmei	450	0.542 (0.316–1.318)	0.63	22.52	13	6.38	Low
Houlong	450	0.209 (0.112–0.354)	0.52	14.04	13	2.46	Low
Tongxiao	540	0.646 (0.391–1.188)	0.53	15.66	16	7.60	Low
Tongluo	540	0.876 (0.467–2.027)	0.44	23.83	16	10.31	Moderate
Yuanli	630	4.125 (3.253–5.467)	1.47	12.87	19	48.53	High
Central							
Houli	540	1.291 (0.764–2.896)	0.84	10.75	16	15.19	Moderate
Wufeng	450	> 10.00	-	-	-	> 117.65	High
Shengang	450	0.826 (0.526–1.742)	0.88	10.59	13	9.72	Low
Hemei	450	> 10.00	-	-	-	> 117.65	High
Lukang	450	> 10.00	-	-	-	> 117.65	High
Southern							
Minxiong	450	> 10.00	-	-	-	> 117.65	High
Liujiao	450	> 10.00	-	-	-	> 117.65	High
Houbi	450	> 10.00	-	-	-	> 117.65	High
Wandan	450	> 10.00	-	-	-	> 117.65	High
Eastern							
Toucheng	630	0.213 (0.154–0.313)	0.95	22.01	19	2.51	Low
Jiaoxi	540	1.328 (0.654–5.171)	0.86	22.67	16	15.63	Moderate
Dongshan	450	> 10.00	-	-	-	> 117.65	High
Yilan	450	> 10.00	-	-	-	> 117.65	High
Sanxing	450	> 10.00	-	-	-	> 117.65	High
Changbin	450	0.108 (0.083–0.141)	1.17	21.31	13	1.27	Low
Taitung	540	1.122 (0.642–2.340)	0.50	10.78	16	13.20	Moderate

[z] Number of *R. dominica* adults tested.
[y] Estimated lethal concentration values with fiducial limits (FL).
[x] Resistance ratio = LC_{50} of each strain divided by LC_{50} of Lab-strain.
[w] Degree of resistance ratio was classified as low for RR ≤ 10, moderate for 10 < RR ≤ 40, high for 40 < RR ≤ 160, and extremely high for RR ≥ 160.

the Lab strain. Five strains were identified in central Taiwan, *R. dominica* populations were classified as moderate resistance to deltamethrin with an LC_{50} of 1.291 mg mL^{-1} in Houli. While strains from Wufeng, Hemei, and Lukang displayed high deltamethrin resistance, with the resistance ratio of greater than 117.65-fold compared with the Lab strain. In southern Taiwan, all strains showed high deltamethrin resistance, with resistance ratios exceeding 117.65-fold compared with the Lab strain. Among 7 strains of *R. dominica* identified in eastern Taiwan, populations from Jiaoxi and Taitung were classified as moderate deltamethrin resistance with the LC_{50} ranging from 1.122 to 1.328 mg mL^{-1}. The *R. dominica* populations were classified as high deltamethrin resistance, with resistance ratios more than 10 mg mL^{-1} in Dongshan, Yilan and Sanxing. In contrast, strains from Toucheng and Changbin were classified as low resistance to deltamethrin, with a resistance ratio of 2.51 and 1.27, respectively.

Overall, 19.0% of the collected strains were moderate resistance to deltamethrin, while 52.4% of collected strains showed high deltamethrin resistance.

DISCUSSION

In this study, *R. dominica* populations from paddy rice warehouses have developed a certain deltamethrin resistance in Taiwan. Of 21 strains tested, 52.4% showed high resistance to deltamethrin, with the highest resistance ratio exceeding 117.65-fold, which is higher than reported by Chen & Chen (2013) at 63.8-fold in 25.0% of populations. It meant that the deltamethrin resistance of *R. dominica* became more serious after 11 years of deltamethrin application. Among these 21 strains, *R. dominica* populations in southern Taiwan showed a high frequency of deltamethrin resistance. This could be attributed to higher temperatures in the south of region compared to other regions, which led to higher reproductive rates of *R. dominica* populations and accelerated the development of insecticide resistance (Edde 2012).

Currently, 3 possible reasons are speculated for the occurrence of deltamethrin resistance in *R. dominica* populations from paddy rice warehouses. First is the long-term use of insecticides with a single mode of action. Between 1992 and 1995, it was successively discovered that *Sitophilus zeamais* Motschulsky and *Corcyra cephalonica* (Stainton) are resistant to the organophosphate phoxim (Kao & Tzeng 1992; Yao & Lo 1994, 1995). Subsequently, experiments conducted in 2000 confirmed that deltamethrin mixed with paddy rice could effectively control *R. dominica* and *S. cerealella* lasting for over a year (Yao & Lo 2000). Therefore, the pyrethroid deltamethrin has been recommended to replace the organophosphate phoxim for controlling stored product pests in paddy rice warehouses since 2002. Even though the organophosphate malathion is another recommended insecticide with the paddy rice-mixing method to control stored product pests, it was discovered that *S. cerealella* collected from Pingtung had developed resistance to malathion, with a resistance ratio of 111-fold (Kao & Tzeng 1992). This finding prompted the Agriculture and Food Agency to purchase only deltamethrin for warehouse managers in Taiwan for 21 years. Consequently, it may lead to the increase of selection pressure from a single insecticide, resulting in the development of *R. dominica* with high resistance to deltamethrin. Second is the incorrect application of deltamethrin DP. Since deltamethrin is a contact-type insecticide, it cannot contact the stored product pests of paddy rice if it is applied only to the exterior of storage bags. This not only fails to control stored product pests effectively, but also increases costs and risk of generating high resistance of stored product pests to deltamethrin. Moreover, insufficient exposure of insects to the insecticide may lead to insecticide-induced hormesis, which increases insect reproduction by enhancing the mating behavior or fecundity at low doses of insecticide treatments (Qu *et al*. 2015; Tuelher *et al*. 2017; Feng *et al*. 2019; Malbert-Colas *et al*. 2020). Exposure of pyrethroid-resistant *S. zeamais* to 0.05 ppm

deltamethrin led to a relatively high net reproductive rate (R_0) and intrinsic rate of increase (r_m), whereas maize weevils treated with 0.5 to 5 ppm of deltamethrin showed relatively low offspring body mass, net reproductive rate (R_0) and intrinsic rate of increase (r_m) (Guedes *et al*. 2010). Outbreaks of stored product pests may occur rapidly after incorrect deltamethrin application due to insecticide-induced hormesis. Third is the transportation of paddy rice between warehouses. Since there is insufficient space for warehouses and processing machinery such as paddy dryers and rice milling machines, some paddy rice may be transferred to other warehouses for storage or processing. This transportation of paddy rice might allow the high deltamethrin-resistant *R. dominica* to spread to other warehouses and lead to the occurrence of deltamethrin resistance for *R. dominica* in the paddy rice warehouses where no insecticides were applied. Therefore, it is necessary to carefully assess the presence of high insecticide-resistant *R. dominica* populations in the original storage location before transporting paddy rice around.

To address the development of deltamethrin resistance in *R. dominica* populations, solutions could include: (1) reducing the frequency of insecticide applications and (2) employing alternative insecticides with different modes of action. Preventing the development of insecticide resistance in pests is more crucial than controlling pests that have already developed high resistance to insecticides. Practicing good sanitation for processing machines such as paddy dryers and rice milling machines and applying non-chemical control materials such as ventilation fans, light traps, pheromones, and natural enemies are the ideal strategies to delay the development of insecticide resistance in insects. (Boyer *et al*. 2012; Edde 2012; Yao *et al*. 2022). Additionally, using alternative insecticides may serve as an effective method to combat the high deltamethrin-resistant lesser grain borer. Currently, insecticides that can potentially control *R. dominica* are categorized into three groups with seven different modes of action (Table 2). The first category of insecticides that are currently registered for mixing with paddy rice in Taiwan includes organophosphate malathion. Malathion was initially recommended for mixing with paddy rice in 1971. However, malathion was replaced by phoxim after the occurrence of malathion resistance in stored product pests in 1979. High malathion-resistant *S. cerealella* was still identified 13 years after 1979 (Kao & Tzeng 1992). After 32 years without malathion application since 1992, the malathion resistance of stored product pests may have declined. The Agriculture and Food Agency could consider providing malathion to warehouse managers for controlling *R. dominica* if a decline of malathion resistance in stored product pests is confirmed, and the pesticide companies would supply it again. The second category of insecticides includes organophosphate phoxim and pyrethroid permethrin, which are recommended for disinfecting empty warehouses. Although this category of insecticides is not used for paddy rice-mixing, but they are registered for controlling the stored product pests in empty warehouses. Therefore, this category of insecticides could potentially be used in paddy rice-mixing, and the pesticides-registration process could be simpler than pesticides that have not been registered in Taiwan. The third category of insecticides is used internationally to control *R. dominica*, but it has not been registered for use in warehouses in Taiwan. This category of insecticides includes pyrethroid phenothrin, spinosyn spinosad, avermectin abamectin, juvenile hormone analogue methoprene, bacterium *Bacillus thuringiensis* subsp. *kurstaki*/*tenebrionis* and fungal agent *Beauveria bassiana* (Mummigatti *et al*. 1994; Beegle 1996; Lord 2005; Oppert *et al*. 2010; Boyer *et al*. 2012; Rumbos *et al*. 2014; Mohamed 2016; Akmal *et al*. 2017; Perišić *et al*. 2020). Spinosad was considered one of the insecticides that can effectively control the lesser grain borer (Fang *et al*. 2002; Chintzoglou *et al*. 2008; Daglish *et al*. 2008; Ramesh Babu *et al*. 2017a). Chen & Chen (2013) confirmed that spinosad was effective in controlling the high

Table 2. The current-registered insecticides that have the potential for controlling *Rhyzopertha dominica* in paddy rice warehouses.

Insecticides[z]	Registered status in warehouses in Taiwan[y]
1B Organophosphates	
Malathion	Recommended for paddy rice-mixing and empty warehouse disinfection
Phoxim	Recommended for empty warehouse disinfection
3A Pyrethroids	
Permethrin	Recommended for empty warehouse disinfection
Phenothrin	Not recommended in warehouses
5 Spinosyns	
Spinosad	Not recommended in warehouses
6 Avermectins & Milbemycins	
Abamectin	Not recommended in warehouses
7A Juvenile hormone analogues	
Methoprene	Not recommended in warehouses
11A *Bacillus thuringiensis*	
B. thuringiensis subsp. *kurstaki*	Not recommended in warehouses
B. thuringiensis subsp. *tenebrionis*	Not recommended in warehouses
UNF Fungal agents	
Beauveria bassiana	Not recommended in warehouses

[z] Insecticide mode of action was classified by the Insecticide Resistance Action Committee (IRAC).
[y] The information on registered insecticides for warehouses was collected from the pesticide information service website (https://pesticide.aphia.gov.tw/information, visited on 2024/06/16) under the Animal and Plant Health Inspection Agency, Ministry of Agriculture, Taiwan.

deltamethrin-resistant *R. dominica* populations collected from Nantou as well as from the 15 strains in Taiwan. However, due to its higher cost than deltamethrin, spinosad was not used in paddy rice warehouses at that time. Methoprene, one of the insect growth regulators, is relatively non-toxic to humans, mammals, and fish. Methoprene does not directly kill insect adults but rather interferes with the development of immature stages, thereby reducing the offspring number. Globally, it is believed that alternating methoprene with deltamethrin or spinosad can effectively control *R. dominica* (Arthur 2004; Athanassiou *et al*. 2011; Arthur 2019). To diversify insecticide options used in paddy rice warehouses, selecting alternative insecticides requires further investigations in Taiwan. In addition to finding alternative insecticides, exploring the resistance mechanisms of *R. dominica* against deltamethrin will be a crucial focus for future research. Understanding these resistance mechanisms may assist in improving pest resistance management strategies in paddy rice warehouses.

Currently, most warehouse managers prefer to apply deltamethrin DP to the exterior of storage bags. This preference likely comes from concerns over the labor-intensive and time-consuming process of rice mixing method, as well as the potential respiratory health risks associated with dust. Therefore, we believe it is necessary to collaborate with agricultural machinery experts to incorporate the paddy rice-mixing method of insecticides into the standard operating procedures for the paddy rice drying process. By doing so, warehouse managers may be more willing to apply insecticide DP appropriately.

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臺灣稻穀倉庫中穀蠹 (鞘翅目：長蠹蟲科) 對第滅寧之感受性評估

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摘要

馮文斌、李啟陽、姚美吉。2025。臺灣稻穀倉庫中穀蠹 (鞘翅目：長蠹蟲科) 對第滅寧之感受性評估。台灣農業研究 74(1):1–10。

穀蠹 (*Rhyzopertha dominica* (Fabricius)) 為我國公糧稻穀倉庫中最常見的主要害蟲，其幼蟲與成蟲均能咬破稻穀的穎殼，直接侵入稻穀內部取食，造成嚴重危害。目前公糧稻穀儲藏期間主要使用 0.055% 第滅寧粉劑與稻穀均勻混拌來防治穀蠹。然而自 2002 年推薦使用第滅寧至今，長期使用單一作用機制的藥劑可能已導致積穀害蟲產生抗藥性。本研究從臺灣各地稻穀倉庫採集 21 個穀蠹族群，並透過稻穀混拌法取得穀蠹對第滅寧的抗藥性數據，結果顯示霧峰、鹿港、民雄、六腳、後壁、萬丹、冬山、宜蘭及三星地區的抗藥性最為嚴重，抗性比最高超過 117.65 倍，證實經過多年連續使用，確實已經導致穀蠹發展出第滅寧抗藥性。為解決因抗藥性引起的第滅寧殺蟲效果下降問題，未來將逐步篩選替代藥劑，以增加我國公糧稻穀倉庫混拌藥劑的種類，減少因抗藥性造成之積穀害蟲防治效果不彰。

關鍵詞：稻穀倉、穀蠹、第滅寧、半致死濃度、抗性比。

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Research Article

Phytochemicals and Physiological Activities of Sweetpotato (*Ipomoea batatas* L.) Leaves Processed via Full Fermentation

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Abstract

Lee, Y. L., C. L. Huang, T. H. Hung, W. T. Liu, S. Y. Lin, C. H. Chen, Y. H. Ho, Y. F. Lee, and C. E. Tseng. 2025. Phytochemicals and physiological activities of sweetpotato (*Ipomoea batatas* L.) leaves processed via full fermentation. J. Taiwan Agric. Res. 74(1):11–23.

Two cultivars of leafy sweetpotato (*Ipomoea batatas* L.), 'Purple Leaf' (PL) and 'Fancy Leaf' (FL), were mixed (at a ratio of 2 : 1) and processed to produce a full-fermented leaf tea (FFLT). Two harvests of the leafy tissues were collected to study their bioactive phytochemicals and physiological activities. The first harvest was conducted in the winter (collected on 2021/11/16), and the second was in the autumn (2022/09/01). The materials of the former were divided into leaf blade and petiole portions. Extracts with room-temperature distilled water (ddH$_2$O) were conducted to examine the total phenolics and flavonoids contents, and the reducing power was analyzed to reveal their antioxidative activities. Results showed that the nutritional values of the leaf blade tissues were much higher than those of the petioles. The FFLT samples made from both harvests were prepared with boiling ddH$_2$O and showed similar reducing power. In contrast, the flavonoid content of the harvest in the autumn was 1.7 times that of the winter, but the latter was slightly higher in the phenolic content than the former. The physiological activity was studied with the FFLT prepared from the second harvest, and its raw material of the leaf tea (RMLT) was also extracted with boiling ddH$_2$O for comparison. Results showed that both FFLT and RMLT extracts exhibited anti-inflammatory activity by reducing nitric oxide (NO) production; however, the latter increased TNF-α production (a detrimental response), while the former did not. In inhibiting protein glycation, both extracts showed effectiveness, with the FFLT being more effective than the RMLT. These results suggest that the full-fermentation manufacturing process converts certain phytochemicals in sweetpotato leaves to enhance their bioactivity, thereby making the resulting leaf tea a potential food with improved health benefits.

Key words: Leafy sweetpotato, Total phenolics, Total flavonoids, Anti-inflammation, Anti-glycation.

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INTRODUCTION

Sweetpotato, *Ipomoea batatas* L., is a popular summer vegetable in Taiwan and also the sixth most important food crop worldwide. In the tropics, this plant is more tolerant to diseases and pests and to a certain level of moisture (waterlogging) than many other leafy vegetables. In Changhua County (the west side of middle Taiwan), sweetpotato leaves can be harvested 24−26 times annually, with an annual yield higher than other green vegetables. It is noted that sweetpotato has been selected as a primary food source by the U.S. National Aeronautics and Space Administration (NASA) because it can be grown in controlled ecological life-support system. Most varieties of sweetpotato are grown for their roots, which are rich in starch and used as staple food or feeds. Nevertheless, its leaves full of abundant phytochemicals, such as polyphenols and dietary fibers, have drawn researchers' attention. In Taiwan, there are cultivars especially bred for leaves consumption as the leaves are palatable after cooking. Compared to many other commercial vegetables, the sweetpotato tops (composed of leaves and stems) contain higher nutritional components such as vitamin A, ß-carotene, iron, calcium, and zinc. Table 1 lists the nutritional value of sweetpotato leafy tissues (including leaf blades and petioles), published by the Ministry of Health and Welfare of Taiwan, ROC.

Islam (2006, 2014) revealed that the nutritional value of sweetpotato leaves is comparable to that of spinach. The high oxalic acid (a leading cause of stone formation in the human body) may be a problem when consuming spinach, but sweetpotato leaves contain less than one-fifth of that in spinach. The leaves are a good source of antioxidative polyphenolics, composed of caffeic acid and five kinds of caffeoylquinic acid derivatives, and, in this regard, they are better than many other commercial vegetables. Islam (2014) examined 82 kinds of vegetable juices and plant components and found that sweetpotato leaves have an overwhelmingly high antimutagenicity; the biologically active compounds were proven to possess multifaceted functions. The animal experiments revealed that the anti-diabetic compounds in sweetpotato leaves reduce the blood glucose level significantly. Besides, the water extract of sweetpotato leaves (from

Table 1. The minerals and nutrients contents[z] of fresh sweetpotato leafy tissue (including petioles).

Analysis item	Unit	Per 100 g serving	*SD*[y]	No. of sample
Minerals				
Sodium (Na)	mg	39	27	5
Potassium (K)	mg	401	82	5
Calcium (Ca)	mg	105	47	5
Magnesium (Mg)	mg	35	17	5
Iron (Fe)	mg	2.5	0.9	5
Zinc (Zn)	mg	0.5	0.1	5
Phosphorus (P)	mg	44	10	5
Copper (Cu)	mg	0.003		1
Total vitamin A	I.U.[x]	5,960		
Retinol equivalent (RE)	μg	596		
α-Carotene	μg	105	148	2
β-Carotene	μg	3,523	2,818	5

[z] Online data of the Ministry of Health and Welfare, Taiwan, ROC.
[y] Standard deviation.
[x] International unit.

the variety 'Simon1') possesses antibacterial activity against many food poisoning bacteria, including *Staphylococcus aureus*, *Bacillus cereus* and lethal *Escherichia coli* O157. The main components in the antibacterial extract are polysaccharides (Islam 2014).

The phytochemicals in the sweetpotato leaves have been identified as phenolics, anthocyanins, and flavonoids (Luo *et al*. 2021; Jia *et al*. 2022) that displayed remarkable anti-oxidant and hypoglycemic activities, both related to anti-aging and anti-diabetes properties. In Taiwan, four cultivars ('TNG10', 'TNG57', 'TNG66', and 'YSP') leaves have been revealed to possess remarkable antioxidative activity, especially in the 'TNG10' and 'TNG57', which couldprotect cells from H_2O_2-induced cytotoxicity (Liao *et al*. 2011). Currently, aging and diabetic complications such as retinopathy, neuropathy, and nephropathy are correlated to the formation of advanced glycation end products (AGEs) (Sano *et al*. 2022), and the inhibition of AGEs formation could prevent these related disorders. To our knowledge, only the roots of sweetpotato have been studied to show a reduced protein glycation level in diabetic rats (Akhtar *et al*. 2018), and the leaf tissue has not yet been studied.

Owing to the high yield of this vegetable in almost all seasons in Taiwan, food processing is a plausible way to endow sweetpotato leaves with a new character on the food market. Black tea made from *Camellia sinica* is a popular drink on the beverage market for a long time. Its fermentation process creates oxidized polyphenolic compounds, such as theaflavins and thearubigins, which are responsible for the color, taste, and aroma of black tea and are beneficial to human health (Jolvis Pou 2016). In this study, we collected two cultivars of leafy sweetpotato, 'Purple Leaf' (PL, Fig. 1A) and 'Fancy Leaf' (FL, Fig. 1B) and processed their mixed-raw materials to produce tea (Fig. 1C) with a black-tea-similar fully fermentation procedure (Zeng & Hong 2020). Experiments were employed to compare the total phenolics, flavonoids, and reducing power. The bioactivities of anti-inflammation and anti-glycation were analyzed. The results showed that the fully-fermented sweetpotato leaf tea might possess these physiological activities and it is worth studying in the future.

MATERIAL AND METHODS

Plant materials

Two leafy sweetpotato cultivars, 'Purple Leaf' (PL, Fig. 1A) and 'Fancy Leaf' (FL, Fig. 1B), were grown at the bank of the Zhuoshui River in Changhua County (GPS 23.824110, 120.444800), Taiwan. Plant materials were collected twice, the first in the winter (on 2021/11/16) and the second in the fall (on 2022/09/01). The winter's raw materials were divided into leaf blade and petiole parts to give an overview on the bioactive phytochemicals. The sweetpotato leaf tea samples were prepared from these two harvests with a full fermentation procedure (Invention Patent TWI680720B, Republic of China. Zeng & Hong 2020). The mixtures of the leaves (without petiole) of PL and FL at a ratio of 2 : 1 were used. The leaf tea was prepared by the following steps: (1) washed the leaves thoroughly; (2) allowed the leaves to air-dry at 3℃ to 25℃ for 6 to 12 h; (3) withered the leaves to reduce moisture content by 5% to 18%; (4) roasted the leaves at temperatures between 100℃ and 350℃, with a batch size of 3 to 12 kg, for 2 to 15 min; (5) rolled the leaves; (6) loosened the rolled leaves; and (7) baked the leaves several times until the moisture content was reduced to below 4%, which was conducted at temperatures of 40℃ to 350℃, with each baking cycle reducing weight by 6% to 26%. In the final baking cycle, the leaves were roasted from 220℃ to 350℃ until a charred aroma emerged (Fig. 1C).

Sample preparation for analysis of phenolics, reducing power, and flavonoids

In the fresh plant materials (moisture content ~ 80%), five leaves with similar appearance (color and size) were selected. Before extraction, the leaf blades and petioles were divided, and ground in liquid nitrogen with

Fig. 1. The tested leafy sweetpotato cultivars included (A) 'Purple Leaf' (PL), (B) 'Fancy Leaf' (FL), and (C) full-fermented leaf tea sample (FFLT) processed at a ratio of 2 : 1 of the leaves PL and FL cultivars.

mortar and pestle to fine powder. An aliquot of room-temperature ddH_2O at a ratio of 20 : 1 (ddH_2O : sample weight) was added and thoroughly mixed to extract the soluble fraction. The sample of fully-fermented leaf tea (FFLT, without grinding) was added to boiling ddH_2O at a ratio of 20 : 1 (ddH_2O : sample weight) and incubated for 20 min for extraction. The extracts were centrifuged at 13,800× *g* for 20 min, and each respective supernatant was collected and filtered through a 0.22-μm PTFE filter (Jet Biofiltration Co., Guangzhou, China) before analyses.

Total phenolics

The method of Singleton & Rossi (1965)

was applied for the determination of total phenolics. A 15-μL extract was mixed well with 240 μL ddH_2O and 15-μL Folin-Ciocalteau phenol reagent (Sigma-Aldrich Co., Buchs, Switzerland) before 30-μL 35% $Na_2CO_{3(aq)}$ (Merck Co., Darmstadt, Germany) was added to the mixture. The mixture was vigorously mixed with a Vortex Mixer before being centrifuged at 16,200× *g* for 2 min. A 200-μL aliquot of the supernatant was measured for the absorbance at 750 nm. Each sample was measured with triplicates. Gallic acid (GA, Acros Organics, China) solution was prepared with ddH_2O as a standard at concentrations of 0.025, 0.05, 0.075, 0.1, 0.2, 0.3, 0.4, and 0.5 mg mL^{-1}.

Reducing power

The method of Oyaizu (1986) was applied to determine the reducing power. A 100-μL sample was added to 100-μL of 0.2 M sodium phosphate buffer (pH 6.6, Merck Co., Darmstadt, Germany) and 100-μL of 1% potassium ferricyanide (Merck Co., Darmstadt, Germany), mixed thoroughly, and incubated at 50℃ for 20 min. The mixture was then cooled down in ice bath and added 100-μL of 10% trichloroacetic acid (Merck Co., Darmstadt, Germany) to stop the reaction. One hundred μL of the mixture was added with 100-μL ddH_2O and 20-μL of 0.1% ferric chloride (Merck Co., Darmstadt, Germany) solution. The mixture was thoroughly mixed, and the absorbance at 700 nm was measured for each sample in triplicate. Trolox standards (0.08, 0.2, 0.4, 0.6 and 0.8 mM) were prepared in methanol to establish a standard curve.

Total flavonoids

Determining total flavonoids followed the method provided by Meda *et al.* (2005). Fifty μL sample was added with 100-μL 2% aluminum trichloride (Alfa Aesar, Ward Hill, MA, USA) prepared in methanol, and the mixture was preserved at room temperature for 10 min before monitoring the absorbance at 415 nm, with each sample in triplicate. The absorbance values of each sample were then subtracted with their absorbance at 415 nm (The 2% aluminum trichloride solution was replaced with methanol solvent.). Quercetin standards (2, 4, 6, 8, 10, 20, 30, 40, and 50 mg L^{-1}) were prepared in methanol to establish a standard curve.

Sample preparation for analysis of anti-inflammation and anti-glycation assays

The sweetpotato leaves of the second harvest (collected on 2022/09/01) were used for the bioactivity assays, inhibition of inflammation and protein glycation. The FFLT sample was prepared as mentioned above. The RMLT (raw material of FFLT) was also prepared to investigate the influences exerted by the full-fermentation process. Ten fresh leaves of each PL and FL cultivars with similar appearance were selected separately. They were ground independently in liquid nitrogen with mortar and pestle to fine powder. An aliquot of boiling ddH_2O at a ratio of 20 : 1 (ddH_2O : sample weight) was added, thoroughly mixed, and incubated for 20 min to extract the soluble fraction. The mixture of PL and FL extracts with 2 : 1 ratio was mixed to serve as the RMLT sample. The extracts were centrifuged at 13,800× *g* for 20 min, and each respective supernatant was collected and filtered through a 0.22-μm PTFE filter (Jet Biofiltration Co., Guangzhou, China) before analyses. To quantify the sample amounts used in the bioactivity assay, the extract's dry weight was applied. Briefly, each 2.00-mL aliquot of the extract was dried in an oven set at 75℃, and the resulting dry weight was used to calculate the liquid weight needed to achieve a dry weight of 100−1,000 μg, individually.

Anti-inflammation assays

Murine macrophage cell line RAW264.7 was purchased from Bioresource Collection and Research Center (BCRC, Hsinchu, Taiwan, R. O. C.). The cells were cultured in DMEM (Dulbecco's Modified Eagle's Medium, Hyclone, New York, USA.) supplemented with 10% fetal bovine serum (Fetal Bovine Serum, FBS, Hyclone, New York, USA) and incubated at 37℃ in an incubator with 5% CO_2. For anti-inflamma-

tion activity assay, the RAW264.7 cells were seeded into 48-well plates at a density of 8 × 10^4 cells per well and cultured for 24 h. These cells were treated with 100 μg dry wt. of the extract or 50 μM hydrocortisone (HC, Sigma-Aldrich Co., Burlington, MA, USA) and 1 μg mL^{-1} of lipopolysaccharide (LPS, Sigma-Aldrich Co., Burlington, MA, USA). After 24 h, each well's supernatant was collected to detect the produced amounts of nitric oxide (NO) and tumor necrosis factor-α (TNF-α). The FFLT sample was tested for NO production analysis at the following dosages: 100, 500, and 1,000 μg (dry wt.). The NO production was determined by Griess Reagent (modified) (Sigma-Aldrich Co., Burlington, MA, USA). Concentrations of TNF-α in supernatants were measured with enzyme-linked immunosorbent assay (ELISA) kits (BioLegend Com., San Diego, CA, USA) according to the manufacturer's instructions.

Anti-glycation assays

The method used was modified by Sano *et al.* (2022). Bovine serum albumin (BSA) 4 mg mL^{-1} was incubated with 200 mM glucose with or without 100 μg extract (dry wt.) in 0.1 M sodium phosphate buffer (pH 7.4) for 7 d at 37℃. After incubation, the level of produced glycated BSA was measured with a glycated BSA-specific ELISA. The horseradish peroxidase (HRP) anti-carboxymethyl lysine antibody (ab27686) was purchased from ABCAM Co. (Cambridge, UK) and the enzyme-linked immunosorbent assays were conducted with the provided instructional protocol. The glycation degree (%) = At/Ac × 100, where At is the level of glycated BSA under co-incubation with the tested sample, and Ac is the level of glycated BSA with the incubation mixture without the tested sample as the control. The analysis was conducted in triplicate.

RESULTS

Total phenolics

The total phenolics of the two leafy sweetpotato cultivars PL and FL samples are shown in Fig. 2. The fresh materials, divided into leaf

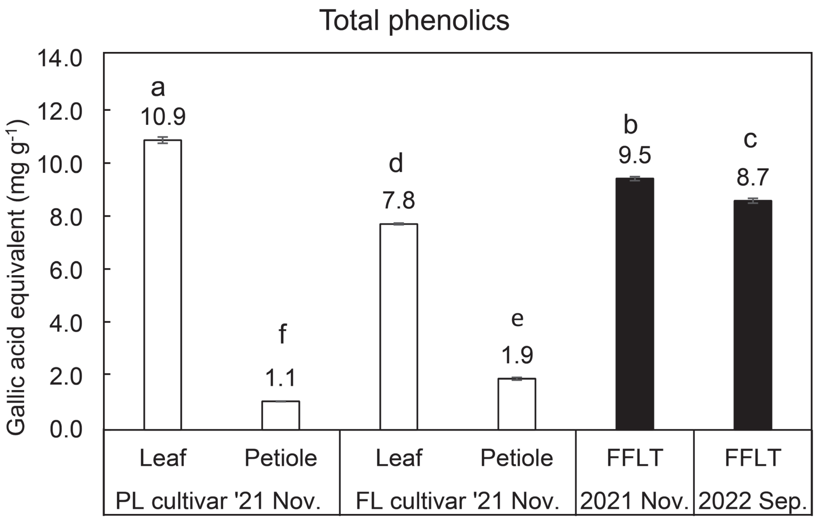

Fig. 2. Total phenolics of samples were extracted with RT (room temperature) ddH_2O (white columns) from fresh materials harvested on 2021/11/16, and with boiling water (black columns) from FFLT samples manufactured in 2021 November and 2022 September, respectively. PL, 'Purple Leaf' cultivar; FL, 'Fancy Leaf' cultivar; FFLT, full-fermented leaf tea. One-way analysis of variance (ANOVA) with Tukey's multiple comparison test (significant $P < 0.05$) was conducted. Mean and standard deviation ($n = 3$) are shown on the column, and different letters indicate statistically significant differences.

blades and petioles (collected on 2021/11/16), were extracted with RT (room temperature) ddH_2O. The leaves of PL and FL bore phenolic contents 10.9 ± 0.12 and 7.8 ± 0.03 mg gallic acid equivalent g^{-1} tissue, respectively, much higher than their petioles 1.1 ± 0.01 and 1.9 ± 0.05 mg gallic acid equivalent g^{-1} tissue, respectively. The FFLT (full-fermented leaf tea) manufactured from the two harvests, the first in 2021 November and the second in 2022 September, prepared with boiling ddH_2O, the total phenolics were 9.5 ± 0.07 and 8.7 ± 0.08 mg gallic acid equivalent g^{-1}, respectively. It is noted that the total phenolics of the tea sample in winter was slightly higher than that in autumn, with a statistically significant difference.

Reducing power

The reducing power of the extracts is shown in Fig. 3, showing a similar tendency to their phenolic contents. The leaf blades of PL 36.6 ± 0.38 and FL 23.1 ± 0.20 mM Trolox equivalent g^{-1} tissue, were much higher than their petioles 4.6 ± 0.07 and 6.2 ± 0.02 mM Trolox equivalent g^{-1} tissue, respectively. The extracts of FFLT manufactured in the winter (2021 November) 37.6 ± 1.25 and in the autumn (2022 September) 38.8 ± 1.96 mM Trolox equivalent g^{-1}; they were similar, without statistically significant difference.

Total flavonoids

The total flavonoids of the extracts are shown in Fig. 4. The blades of PL 2.30 ± 0.03 and of FL 0.93 ± 0.04 quercetin equivalent mg g^{-1} tissue were much higher than their petioles 0.11 ± 0.004 and 0.17 ± 0.008 mg quercetin equivalent g^{-1}, respectively. The extracts of FFLT manufactured in the winter (2021 November) were 1.05 ± 0.05 and in the autumn (2022 September) 1.80 ± 0.10 mg quercetin equivalent g^{-1}; the latter 1.7 times higher than the former, with a statistically significant difference.

Anti-inflammatory activity

The anti-inflammatory activity of the FFLT and the RMLT (raw material of leaf tea) extracted by boiling ddH_2O were determined with the productions of NO and TNF-α of the RAW264.7 cells (Fig. 5). The inflammatory response was tested by mixing lipopolysac-

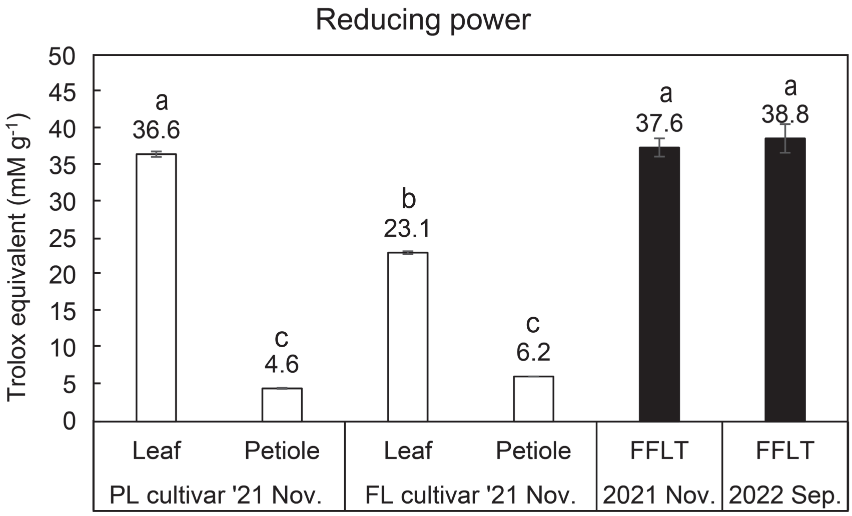

Fig. 3. Reducing powers of samples were extracted with RT (room temperature) ddH_2O (white columns) from fresh materials harvested on 2021/11/16, and with boiling water (black columns) from FFLT samples manufactured in 2021 November and 2022 September, respectively. PL, 'Purple Leaf' cultivar; FL, 'Fancy Leaf' cultivar; FFLT, full-fermented leaf tea. One-way analysis of variance (ANOVA) with Tukey's multiple comparison test (significant $P < 0.05$) was conducted. Mean and standard deviation ($n = 3$) are shown on the column, and different letters indicate statistically significant differences.

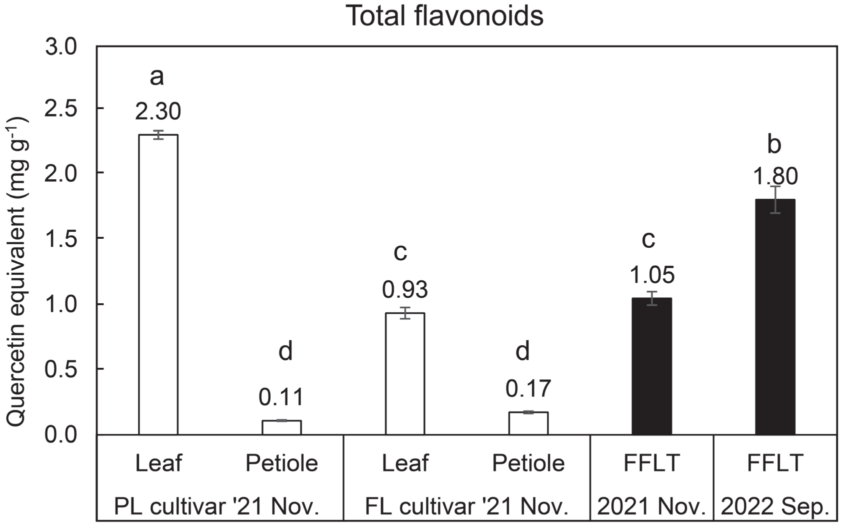

Fig. 4. Total flavonoids of samples were extracted with RT (room temperature) ddH_2O (white columns) from fresh materials harvested on 2021/11/16, and with boiling water (black columns) from FFLT samples manufactured in 2021 November and 2022 September, respectively. PL, 'Purple Leaf' cultivar; FL, 'Fancy Leaf' cultivar; FFLT, full-fermented leaf tea. One-way analysis of variance (ANOVA) with Tukey's multiple comparison test (significant $P < 0.05$) was conducted. Mean and standard deviation ($n = 3$) are shown on the column, and different letters indicate statistically significant differences.

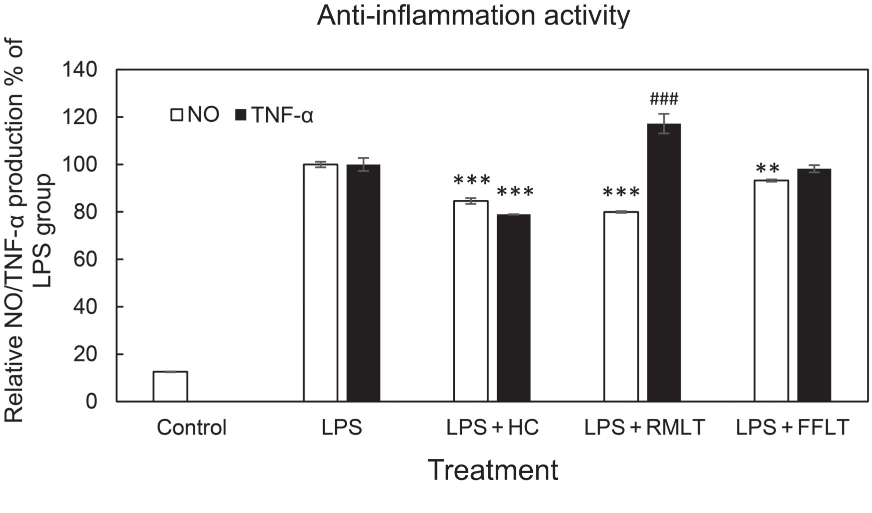

Fig. 5. Bioactivity assay of anti-inflammation with inhibition of nitric oxide (NO) and TNF-α productions. The extract of RMLT and FFLT was applied with 100 μg (dry wt.), white columns representing the NO production and black columns the TNF-α production of RAW264.7 cells, respectively. Lipopolysaccharide is abbreviated to LPS; HC, hydrocortisone; RMLT, raw material of leaf tea; FFLT, full-fermented leaf tea. The analyses were conducted with triplication and the standard deviations shown as error bars ($^{**}P < 0.01$, $^{***/\#\#\#}P < 0.001$ vs. LPS based one-way analysis of variance (ANOVA); * lower, $^{\#}$ higher).

charide (LPS), the inflammation inducer, and the extracts simultaneously, whereas the anti-inflammatory drug hydrocortisone (HC) was used as a positive control. Both FFLT and RMLT effectively reduced the NO production (93.2 ± 0.5% and 80.0% ± 0.4%, respectively,

white columns in Fig. 5) with statistically significant differences. However, they did not inhibit the TNF-α production, especially RMLT significantly enhancing the TNF-α production (122% ± 4%, black columns in Fig. 5). Nevertheless, FFLT showed no change on the TNF-α production (102% ± 2%, without detrimental effect). Different doses of the FFLT extract were further tested with NO production assay. Results (Fig. 6) indicated that the NO production amounts were reduced to 73%, 71%, and 64% in correspondence to FFLT doses of 100, 500, and 1,000 μg, respectively. Therefore, the FFLT extract exhibited a dose-dependent efficacy on the inhibition of NO production.

Anti-glycation of BSA

The anti-glycation of BSA was conducted with the FFLT and RMLT extracts and the results are shown in Fig. 7. Both extracts effectively reduced the glycation degree, and the FFLT showed a higher inhibition activity with an 80% ± 3% glycation degree of the control, better than that of the RMLT (91% ± 3% glycation of the control) with statistically significant difference ($P < 0.05$).

DISCUSSION

Phytochemicals/nutritional value of fresh sweetpotato leaves

In this study the phytochemicals in the fresh leaf and petiole tissues from 2 sweetpotato cultivars, PL and FL, were compared, and the results showed that the phenolics and flavonoids, and reducing powers, were remarkably higher in the leaves than those in the petioles (Figs. 2−4). Jia *et al*. (2022) compared four different aerial parts of leafy sweetpotato and showed the same results. The four parts were buds, leaves, petioles, and stems. Among them, the buds and

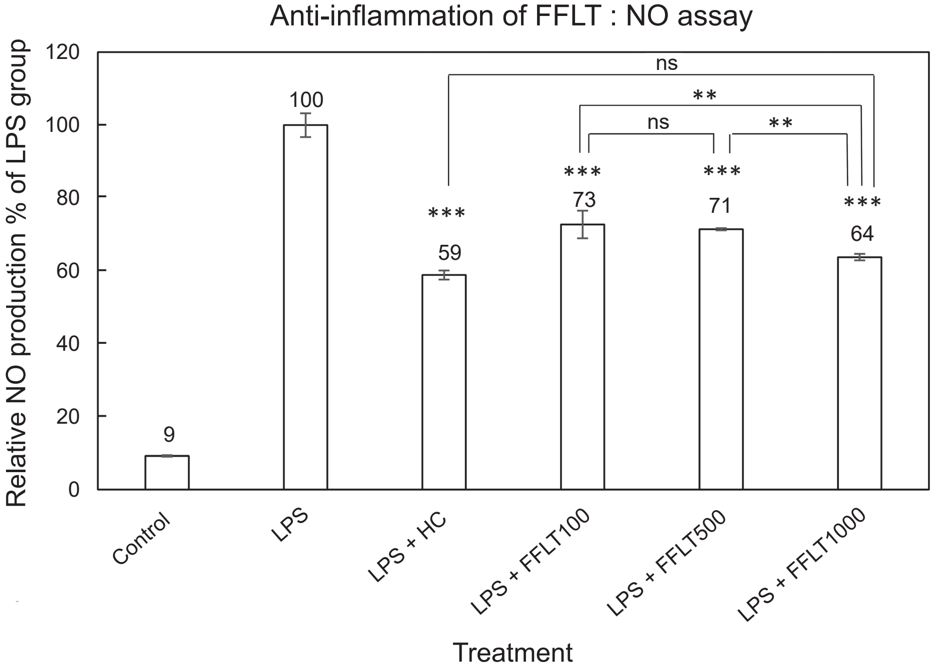

Fig. 6. Anti-inflammation activity of the FFLT sample, with boiling ddH_2O extraction, was determined by nitric oxide (NO) production of RAW264.7 cells. Lipopolysaccharide is abbreviated to LPS; HC, hydrocortisone; FFLT, full-fermented leaf tea; 100, 500, 1,000 were representations of "μg" dry wt. extracts of them, respectively. The analyses were conducted with triplication and the standard deviations shown as error bars ($^{**}P < 0.01$, $^{***}P < 0.001$ vs. LPS based on one-way analysis of variance (ANOVA); with pair comparison, ns is no significant difference).

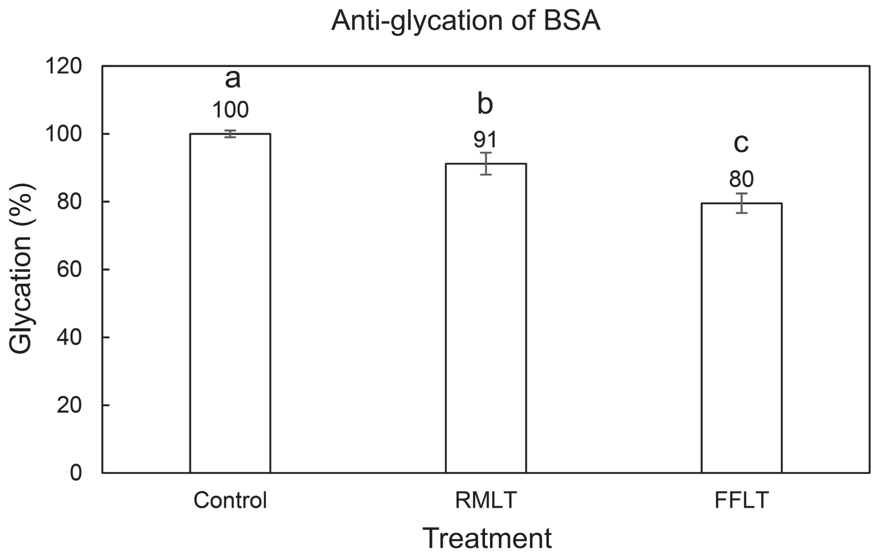

Fig. 7. Anti-glycation of BSA. The extract of RMLT and FFLT was applied with 100 μg (dry wt.). Glycation degree (%) = At/Ac × 100, where At is the level of glycated BSA under co-incubation with tested sample, and Ac the level of glycated BSA with the incubation mixture without the tested sample as the control. RMLT, raw material of leaf tea; FFLT, full-fermented leaf tea. One-way analysis of variance (ANOVA) with Tukey's multiple comparison test (significant $P < 0.05$) was conducted. Mean and standard deviation ($n = 3$) are shown on the column, and different letters indicate statistically significant differences.

leaves had higher levels of nutritional value, including total phenolics, total anthocyanins, and antioxidative activities. Luo *et al*. (2021) analyzed the phenolics and flavonoids contents of the sweetpotato leaf with ultra-high-performance liquid chromatography and identified 13 individual phenolic acids and 10 individual flavonoids; the former consisted mainly of 8 isomeric caffeoylquinic acids and the latter mainly chrysin, rutin, hyperoside, and pectolinarigenin. All these phytochemicals are strong antioxidative agents in natural plants.

The FFLT made from the two harvests, raw materials collected in 2021 November and in 2022 September, exhibited similar reducing power (Fig. 3). However, the flavonoid content of the latter (the autumn collection) was 1.7 times of those in the former (the winter collection) (Fig. 4), although the former slightly higher in the phenolic content than the latter (Fig. 2). Kobayashi *et al*. (2019) revealed the seasonal variation of the sweetpotato foliage in the yield and polyphenol content with 3 consecutive years in a greenhouse in Japan. They found that the polyphenol content was negatively related to the yield as well as the air temperature. The polyphenol content was higher in the harvests of May and November, and lower during June to August (in the summer, with a higher yield comparatively). It is consistent with our results that the total phenolics in the winter FFLT were slightly higher than those in the autumn (Fig. 2). Nevertheless, their reducing powers were alike (Fig. 3), their total flavonoids contents were opposite, and the autumn FFLT was significantly higher (Fig. 4). The seasonal variation of plant materials is related to the environment and plant physiological conditions. It is worth studying the difference and realizing suitable seasons of the sweetpotato leaves for manufacturing.

Physiological activity- anti-inflammation and anti-glycation activities of FFIT

Anti-inflammation activity was determined by the murine macrophage cell line RAW264.7.

The production of NO or TNF-α represents the cells undergoing an inflammatory response. The results showed both FFLT and RMLT extracts exhibiting anti-inflammation activity for they reduced NO production rates (white columns in Fig. 5); however, the RMLT increased the TNF-α production (black columns in Fig. 5), and the FFLT did not. Additionally, the FFLT exhibited a dose-dependent effect in the NO assay (Fig. 6). This indicates that the manufacturing process of the FFLT altered the bioactive components of sweetpotato leaves, enhancing their potency as an anti-inflammatory agent.

Wu & Yen (2005) studied different stages of protein glycation. Several flavonoids (luteolin, quercetin, and rutin) were identified to inhibit HbA1C formation ($P < 0.01$), with more effectiveness than aminoguanidine (AG, 10 mM, a well-known inhibitor of AGEs). Luteolin and rutin showed significantly high inhibitory capacity in the middle stage on methylglyoxal-medicated protein modification, with the IC50's at 66.1 and 71.8 μM, respectively. In the final stage of glycation, luteolin was found to be a potent inhibitor that could effectively suppress both the formation of AGEs and the subsequent cross-linking of proteins. Additionally, Sano *et al*. (2022) reported that lignin, the major ingredient of a corn silk water extract, was an active component that could inhibit the glycation of BSA. It is plausible that the dominant bioactive compounds in the sweetpotato leaves were chlorogenic acid derivatives, which are lignin precursors. The FFLT was more potent than the RMLT in inhibiting protein glycation (Fig. 7). In this regard, it is suggested that the active components of sweetpotato leaves were transformed through the full fermentation process to exert enhanced anti-glycation activity.

Full fermentation process of sweetpotato leaves converts a certain level of phytochemicals

Similar to the fully fermented black tea made from *C. sinica*, the fermentation of the sweetpotato leaves create a different character for the beverage with brownish color, light sweet taste, and aromatic flavor. The manufacturing procedure of the FFLT (Zeng & Hong 2020) was modified from the traditional black tea production method. Black tea was originated from unfermented green tea. Catechins account for 90% of the phenolic fraction in green tea, whereas only about 15% of catechins remain unoxidized in black tea (Skotnicka *et al*. 2011). Theaflavins and thearubigins belong to the oxytheotannins. Seven different brands of black tea (marketed in England) showed a similar combination of polyphenols dominated by thearubigins (75−82% of total phenolics) (Rechner *et al*. 2002). This indicates the fermentation procedure oxidized the catechins before being aggregated into thearubigins (Skotnicka *et al*. 2011). Similarly, the manufacturing process of the FFLT might result in the production of considerable bioactive components through oxidation and aggregation reactions of the phytochemicals.

Therefore, it is suggested that the full-fermentation manufacturing process transforms the bioactive components of sweetpotato leaves, resulting in more potent anti-inflammatory and anti-glycation activities. This transformation enhances the potential of the resulting leaf tea as a health-promoting product, making it a promising option with advanced benefits. In the future, it is worthwhile to investigate the active components in the FFLT and to study the seasonal variation of phytochemicals in sweetpotato leaves for further applications.

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全發酵甘藷葉茶之植化素與生理活性分析

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摘要

李雅琳、黃哲倫、洪子桓、劉威廷、林素月、陳秋樺、何奕漢、李穎甫、曾崇恩。2025。
全發酵甘藷葉茶之植化素與生理活性分析。台灣農業研究 74(1):11–23。

採用 2 種葉用甘藷品種「紫葉」 (‘Purple Leaf’; PL)、「彩葉」 (‘Fancy Leaf’; FL) 取其葉片 (2 : 1 比例) 混合製成一種全發酵葉茶 (full-fermented leaf tea; FFLT)。兩批試驗原料分別採集於 2021 年冬天 (11 月 16 日) 與 2022 年秋天 (9 月 1 日)。第一批冬天材料將樣品區分為葉片與葉柄，以室溫之蒸餾水萃取其植化素，進行總酚與黃酮類化合物含量分析，並分析其還原力以探討其抗氧化能力，結果顯示葉片營養成分高於葉柄。比較此二批原料製成的葉茶，以沸騰熱蒸餾水萃取其植化素，結果顯示其還原力相近，然而第二批秋天採收製成的葉茶中，黃酮類化合物含量為冬天 (第一批原料) 葉茶的 1.7 倍，但是總酚含量則是後者略高。葉茶生理活性的研究，是使用第二批採收的秋天材料，並以葉茶生原料 (raw material of leaf tea; RMLT) 做為比較；同樣以沸騰熱蒸餾水萃取生原料，分析結果顯示 FFLT 與 RMLT 均具有抑制一氧化氮 (nitric oxide; NO) 產生的抗發炎效果，但是後者有增加 TNF-α 產生的發炎反應，而前者則沒有。針對抑制蛋白質醣化的效果，分析顯示兩者均有效，但 FFLT 勝於 RMLT。由這些結果推測，此全發酵製程使甘藷葉中的某些植化素轉變，從而提升其生理活性，使葉茶成為一種具潛力的健康食物。

關鍵詞：葉用甘藷、總酚、黃酮類化合物、抗發炎、抗醣化。

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台灣農業研究 (J. Taiwan Agric. Res.) 74(1):25–36 (2025)
https://doi.org/10.6156/JTAR.202503_74(1).0003

Research Article

A List of Diaspididae in the Insect Collection of Taiwan Agricultural Research Institute (TARI) (4): Fioriniinae (Hemiptera: Coccoidea: Diaspididae)

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Abstract

Chen, S. P., J. Y. Wong, and H. C. Ku. 2025. A list of diaspididae in the insect collection of Taiwan Agricultural Research Institute (TARI) (4): Fioriniinae (Hemiptera: Coccoidea: Diaspididae). J. Taiwan Agric. Res. 74(1):25–36.

A list of 20 species in 3 genera of Fioriniinae (Hemiptera: Coccoidea: Diaspididae) with 145 slide-mounted specimens deposited at the Insect Collection of the Taiwan Agricultural Research Institute (TARI) is provided. The specimens were mainly collected during the Japanese Colonial Period. Synonyms, host plants, location data, and photographs of slide-mounted specimens are also provided for each species.

Key words: Fioriniinae, Takahashi, Taiwan.

INTRODUCTION

Scale insects (Coccoidea) of Taiwan have been extensively studied by several Western and Japanese entomologists, such as Green, Ferris, and Takahashi, since 1923 (Tao 1978). This is the fourth report of the Diaspididae catalogue series at the TARI. The first three reports treated Aspidiotinae, Chionaspinae, and Diaspidinae (Chen *et al*. 2013, 2014, 2016). Most of the slide-mounted specimens of Fioriniinae (Diaspididae) collected by Takahashi were deposited at the Insect Collection, Division of Applied Zoology, Taiwan Agricultural Research Institute (TARI), including several types. However, many species names used by Takahashi have been changed or synonymized with other names. In this study, the taxonomic status of all slide-mounted specimens in TARI was examined and revised based on the current studies.

MATERIALS AND METHODS

A total of 145 slide-mounted specimens were examined by using a Leitz DMRB light microscope (Leica, Milton Keynes, UK) in this study. Location data of each species were compiled based on the information provided on the slide-mounted specimens deposited at the TARI Insect Collection. Photographs of slide-mounted specimens (adult female) were taken by using a Leitz DMRB light microscope, Automatic Piezo stepping motor (Leica Piezo Z-drive, Leica, Glattbrugg, Switzerland), and Leica DFC 420 Digital Microscope Cameras (Leica, Glattbrugg, Switzerland) for each species. The spelling of localities followed according to Chu & Yamanaka (1973, 1974, 1975).

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RESULTS

Among the 20 species (3 genera) of Fioriniinae in TARI, there were 8 species described by Takahashi, 5 species by Maskell, 3 species by Green, 2 species by Kuwana, and 2 species by Cockerel and Targioni-Tozzetti, respectively. Five species were not collected from Taiwan, including *Fiorinia payaoensis* Takahashi, *Fiorinia pinicola* Maskell, *Unachionaspis bambusae* (Cockerell), *Unachionaspis signata* (Maskell), and *Unachionaspis tenuis* (Maskell). The following list provides comprehensive information on each species' nomenclature, synonymies, host plants, and distribution. Type specimens (female) with deposited labels (for example, #27-4-2-1) were recorded. Table 1 is a checklist of the old name and current names of collection locality records included in the paper (Chu & Yamanaka 1973, 1974, 1975).

Fiorinia ficicola (Takahashi, 1931) (Fig. 1A) (Takahashi 1931b; Takagi 1970; Chou 1982; Tao 1989, 1999; García Morales *et al*. 2016; Normark *et al*. 2019)

Original spelling on the label: *Fiorinia ficicola* Takah. (7 slides)

Location data on the slides: Taiwan (Taihoku: Shinten).

Host plant recorded on the label: *Ficus foveolata* (Moraceae).

Syn.: *Adiscofiorinia ficicola* Takahashi, 1931; *Ichthyaspis ficicola* (Takahashi, 1931)

Note: Syntypes, female (collected from Taiwan: Taihoku, Shinten, on *Ficus foveolata*, 14-VI-1931, by R. Takahashi (#27-2-3-1~5)).

Fiorinia fioriniae (Targioni Tozzetti, 1867) (Fig. 1B) (Targioni Tozzetti 1867; Cockerell 1893; Borchsenius 1966; Tao 1989, 1999; García Morales *et al*. 2016; Liu *et al*. 2020)

Original spelling on the label: *Fiorinia fioriniae* Targ. (21 slides)

Location data on the slides: Taiwan (Heito: Kuraru; Kagi; Taihoku: Maruyama, Shirin, Taihoku; Taito: Seisui; Takao; Urai); Thailand (Mt. Sutep).

Host plant recorded on the label: *Cinnamomum* sp., *Machilus* sp. (Lauraceae); *Eucalyptus* sp. (Myrtaceae); *Ficus* sp. (Moraceae); Mango, *Buchanania sessilifolia* (Anacardiaceae); *Thea japonica* (Theaceae).

Syn.: *Diaspis fioriniae* Targioni Tozzetti, 1867; *Chermes arecae* Boisduval, 1868; *Fiorinia camelliae* Comstock, 1881; *Uhleria camelliae* (Comstock, 1881); *Uhleria fioriniae* (Targioni Tozzetti, 1867); *Fiorinia fioriniae* (Targioni Tozzetti, 1867); *Fiorinia palmae* Green, 1896; *Parlatoria fioriniae* (Targioni Tozzetti, 1867); *Parlatoreopsis camelliae* (Comstock, 1881).

Fiorinia formosensis Takahashi, 1933 (Fig. 1C) (Takahashi 1933; Chou 1982; Tao 1989, 1999; García Morales *et al*. 2016; Liu *et al*. 2020)

Original spelling on the label: *Fiorinia formosensis* Takahashi, 1933. (7 slides)

Location data on the slides: Taiwan (Taihoku: Kanko, Shinten).

Host plant recorded on the label: *Ficus* sp. (Moraceae).

Note: Syntypes, female (collected from Taiwan: Shinten, on *Ficus* sp., 05-IX-1932, by R. Takahashi (#27-4-2-1~27-4-3-2)).

Fiorinia hisakakii Takahashi, 1936 (Fig. 1D) (Takahashi 1936, 1956; Borchsenius 1966; Tao 1989, 1999; García Morales *et al*. 2016; Liu *et al*. 2020)

Original spelling on the label: *Fiorinia vaccini* var. *hisakakii* Takahashi, 1936. (3 slides)

Location data on the slides: Taiwan (Takaoshu: Chushinron, Chushinron near Rokki)

Host plant recorded on the label: *Eurya* sp. (Theaceae).

Syn.: *Fiorinia vaccini hisakakii* Takahashi, 1936; *Fiorinia euryae hisakaki* Takahashi, 1956.

Note: Syntypes, female (collected from Taiwan: Takao, Chushinron, near Rokki, on *Eurya* sp., 19-V-1935 by R. Takahashi (#29-5-2-1~29-5-2-3)).

Table 1. A checklist of the old name, current name and Chinese name of collection localities included in the paper.

Old name	Current name	Chinese name
Chushinron near Rokki	Kaohsiung: Paoshan near Liouguei	高雄：寶山 近 六龜
Dakusui	Yilan: Zhuoshui (Datong)	宜蘭：大同：濁水
Habon	Nantou: Holuan	南投：合巒
Heito	Pingtung	屏東
Hinokiyama	New Taipei City: Kuaishan	新北市：檜山
Hori, Horin	Nantou: Puli	南投：埔里
Hsintien	New Taipei City: Xindian (Sindian)	新北市：新店
Inrin	Changhua: Yuanlin	彰化：員林
Kagi	Chiayi	嘉義
Kaho	Taoyuan: Hsiapu	桃園：下埔
Kahodai	Taichung: Jiabaotai (Chiapaotai)	臺中：佳保台
Kakayo, Kakayom	Taitung: Jiadai (Chiachiadai)	臺東：嘉代 (嘉嘉代)
Kanko	New Taipei City: Gangou (Kankou)	新北市：乾溝
Kinnos near Nano	Yilan: Chihoau	宜蘭：基戛央
Kuraru	Pingtung: Sheding	屏東：社頂
Maruyama	Taipei: Yuanshan	臺北：圓山
Mato	Tainan: Madou	臺南：麻豆
Musha	Nantou: Wushe	南投：霧社
Mutosan	Pingtung: Wutoushan	屏東：霧頭山
Rirei near Kurasu	Taichung: Lileng near Sungho	臺中：裡冷 近 松鶴
Seisui	Taitung: Qingshui (Chingshui)	臺東：清水
Shin-Taiheizan	Yilan: Taiping Mountain (Taipingshan)	宜蘭：太平山
Shinten	New Taipei City: Xindian (Sindian)	新北市：新店
Shirin	Taipei: Shilin	臺北：士林
Shoka	Changhua	彰化
Sozan	Taipei: Yangmingshan	臺北：陽明山
Suisha	Nantou: Shuishe	南投：魚池：水社
Taichu	Taichung	臺中
Taiheizan, Taihezan, Taiheisan	Yilan: Taiping Mountain (Taipingshan)	宜蘭：太平山
Taihoku	Taipei	臺北
Tainan	Tainan	臺南
Taito, Taitocho	Taitung	臺東
Takao, Takaoshu	Kaohsiung	高雄
Toran	Taitung: Dulan (Doulan)	臺東：都蘭
Urai	New Taipei City: Wulai	新北市：烏來

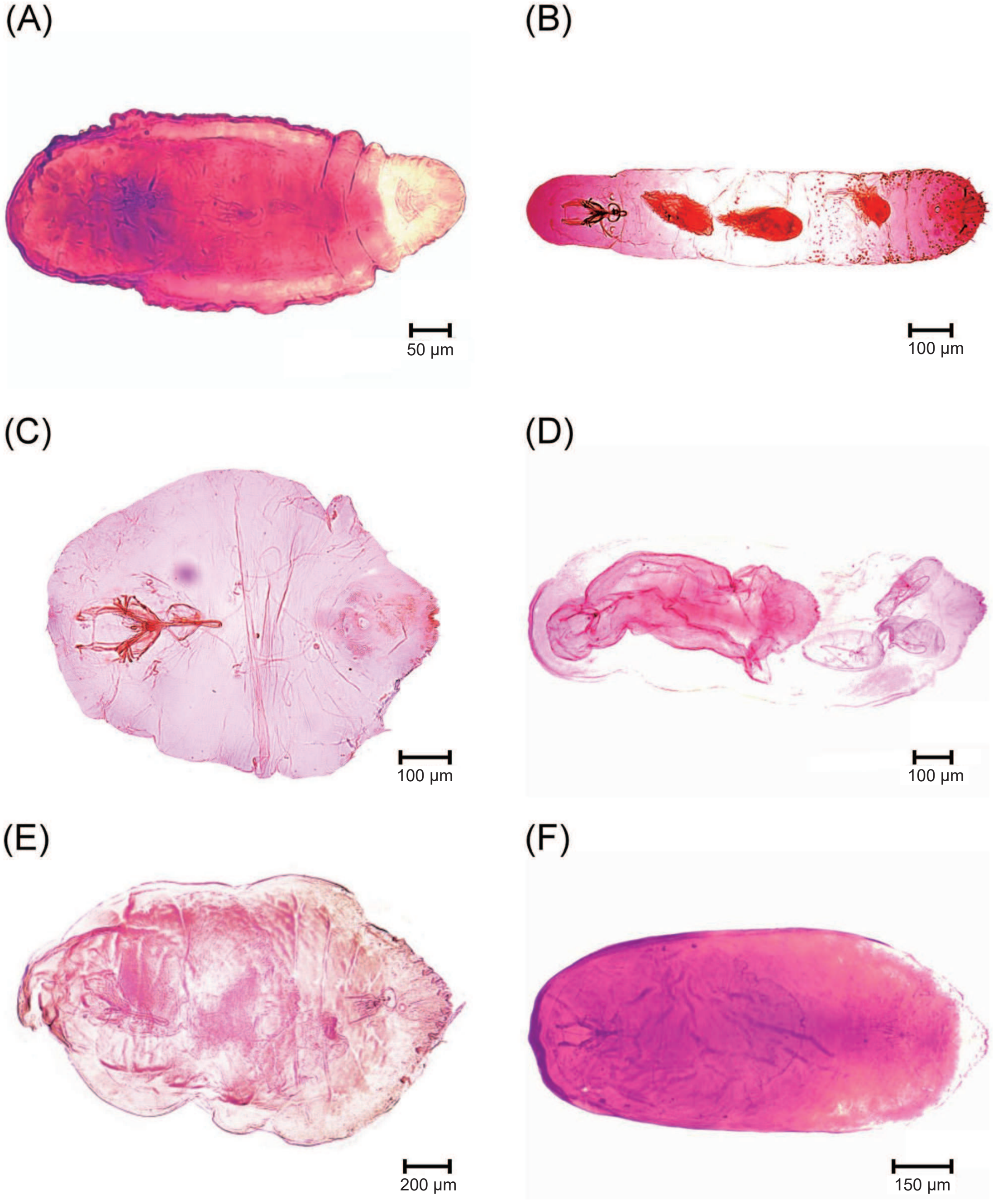

Fig. 1. (A) *Fiorinia ficicola* (Takahashi, 1931); (B) *Fiorinia fioriniae* (Targioni Tozzetti, 1867); (C) *Fiorinia formosensis* Takahashi, 1933; (D) *Fiorinia hisakakii* Takahashi, 1936; (E) *Fiorinia horii* Kuwana, 1927; and (F) *Fiorinia japonica* Kuwana, 1902.

Fiorinia horii Kuwana, 1927 (Fig. 1E)
(Kuwana 1927; Tao 1989, 1999; García Morales *et al*. 2016; Liu *et al*. 2020)

Original spelling on the label: *Fiorinia horii* Kuwana. (3 slides)

Location data on the slides: Japan (Honshu: Tokyo); Taiwan (Nantou: Hori; Taichu: Rirei near Kurasu).

Host plant recorded on the label: *Hymenanthes* sp., *Rhododendron* sp. (Ericaceae).

Fiorinia japonica Kuwana, 1902 (Fig. 1F)
(Kuwana 1902; Ferris 1921; Tao 1989, 1999; Wong *et al*. 1999; García Morales *et al*. 2016; Liu *et al*. 2020)

(1) **Original spelling on the label:** *Fiorinia fioriniae japonica* Kuw. (2 slides)
Location data on the slides: Taiwan (Taihoku).

(2) **Original spelling on the label:** *Fiorinia japonica* Kuwana. (9 slides)
Location data on the slides: Taiwan (Taihoku: Maruyama, Taihoku, Taipei).

(3) **Original spelling on the label:** *Fiorinia juniperi* Leon. (4 slides)
Location data on the slides: Japan (Okinawa: Naha); Taiwan (Taihoku; Tainan).

Host plant recorded on the label: *Pinus* sp., *Keteleeria* sp. (Pinaceae); *Podocarpus* sp. (Podocarpaceae).

Syn.: *Fiorinia fioriniae japonica* Kuwana, 1902; *Fiorinia juniperi* Leonardi, 1906.

Fiorinia minor Maskell, 1897 (Fig. 2A)
(Maskell 1897b; Ferris 1921; Tao 1989, 1999; García Morales *et al*. 2016; Liu *et al*. 2020)

Original spelling on the label: *Fiorinia chinensis* Ferris. (9 slides)

Location data on the slides: Taiwan (Giran: Dakusui, Kinnos near Nano; Taihoku).

Host plant recorded on the label: *Celtis* sp. (Ulmaceae); *Ficus* sp. (Moraceae).

Syn.: *Fiorinia camelliae minor* Maskell, 1897; *Fiorinia fioriniae minor* (Maskell, 1897); *Fiorinia chinensis* Ferris, 1921.

Fiorinia payaoensis Takahashi, 1942 (Fig. 2B)
(Takahashi 1942; Borchsenius 1966; García Morales *et al*. 2016; Liu *et al*. 2020)

Original spelling on the label: *Fiorinia payaoensis* Takah. (6 slides)

Location data on the slides: Thailand (Paiao; Paiyao; Payao).

Note: Syntypes, female (Thailand: Payao, north of Lampang, on undetermined tree, 16-IV-1940 by R. Takahashi (#29-1-1-1~29-1-2-1)).

Fiorinia pinicola Maskell, 1897 (Fig. 2C)
(Maskell 1897a; Borchsenius 1966; Tao 1989; García Morales *et al*. 2016; Liu *et al*. 2020)

Original spelling on the label: *Fiorinia pinicola* Maskell. (1 slide)

Location data on the slides: Japan (Hokkaido: Sapporo).

Host plant recorded on the label: *Abies sachalinensis* (Pinaceae).

Syn.: *Fiorinia camelliae* (Maskell, 1898) (incorrect synonymy); *Fiorinia pruinosa* Ferris, 1950; *Fiorinia junipericola* Borchsenius, 1966.

Fiorinia proboscidaria Green, 1900 (Fig. 2D)
(Borchsenius 1966; Tao 1989, 1999; García Morales *et al*. 2016; Liu *et al*. 2020)

Original spelling on the label: *Fiorinia proboscidaria* Green. (8 slides)

Location data on the slides: Taiwan (Heito: Kuraru; Kagi; Shoka: Inrin; Taito: Kakayo).

Host plant recorded on the label: *Citrus* sp., *Citrus tachibana*, Lemon (Rutaceae); *Syzygium jambos* (Myrtaceae).

Fiorinia randiae Takahashi, 1934 (Fig. 2E)
(Takahashi 1934; Borchsenius 1966; Tao 1989, 1999; García Morales *et al*. 2016; Liu *et al*. 2020)

Original spelling on the label: *Fiorinia randiae* Takah. (3 slides)

Location data on the slides: Taiwan (Nantou: Hori, Suisha)

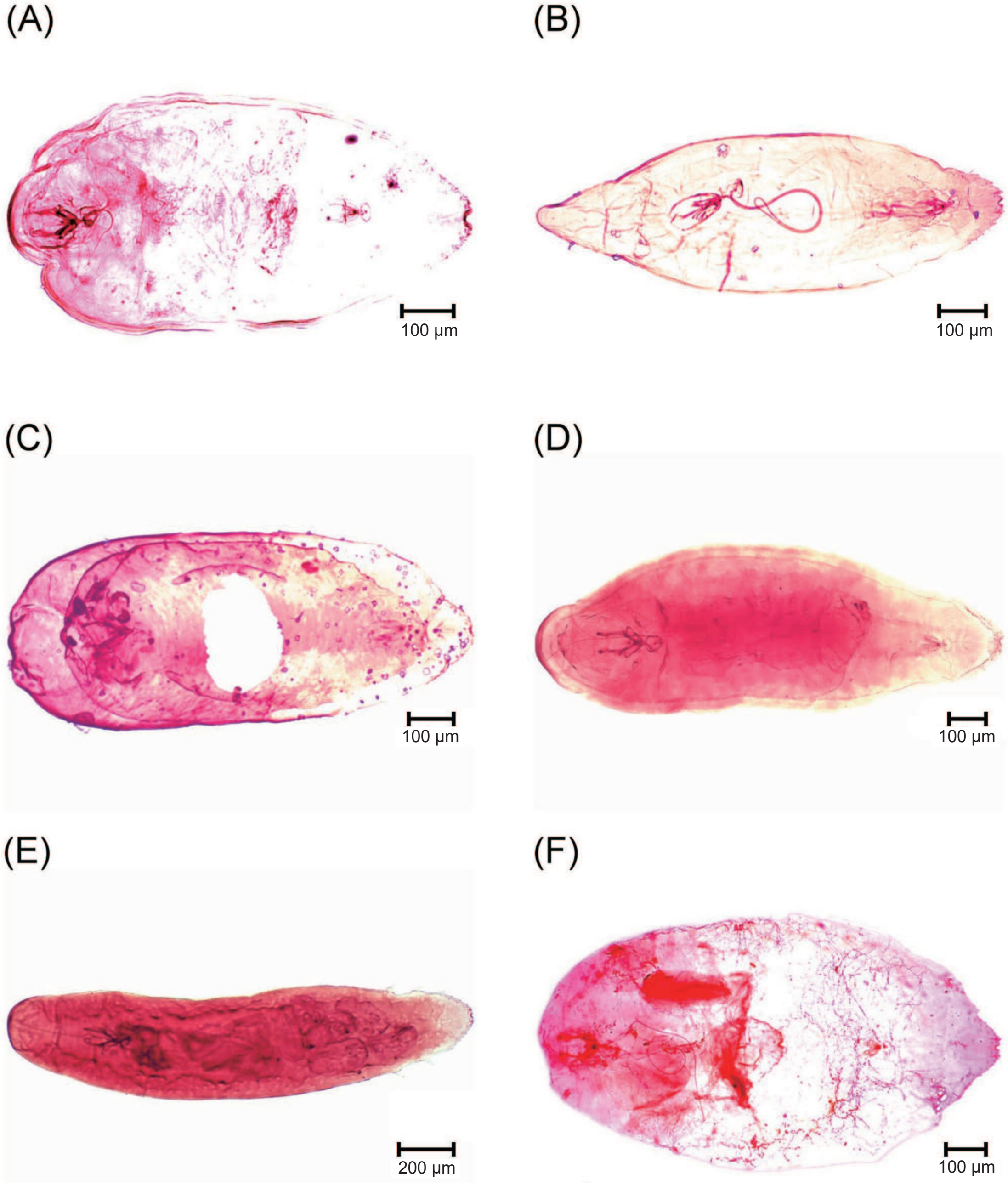

Fig. 2. (A) *Fiorinia minor* Maskell, 1897; (B) *Fiorinia payaoensis* Takahashi, 1942; (C) *Fiorinia pinicola* Maskell, 1897; (D) *Fiorinia proboscidaria* Green, 1900; (E) *Fiorinia randiae* Takahashi, 1934; and (F) *Fiorinia saprosmae* Green, 1896.

Host plant recorded on the label: *Randia canthioides* (Rubiaceae).

Syn.: *Fiorinia proboscidaria randiae* Takahashi, 1934.

Note: Syntypes, female (collected from Taiwan: Hori, on *Randia canthioides*, 10-VI-1933, by R. Takahashi (#29-3-1-1~2)).

Fiorinia saprosmae Green, 1896 (Fig. 2F)

(Green 1896; MacGillivray 1921; Borchsenius 1966; García Morales *et al*. 2016; Liu *et al*. 2020)

Original spelling on the label: *Fiorinia saprosmae* Green. (5 slides)

Location data on the slides: Sri Lanka (Ceylon); Taiwan (Taito: Kotosho).

Host plant recorded on the label: *Ficus* sp. (Moraceae).

Syn.: *Trullifiorinia saprosmae* (Green, 1896).

Fiorinia smilaceti Takahashi, 1931 (Fig. 3A)

(Takahashi 1931a; Borchsenius 1966; Tao 1989, 1999; García Morales *et al*. 2016; Liu *et al*. 2020)

Original spelling on the label: *Fiorinia smilaceti* Tak. (13 slides)

Location data on the slides: Taiwan (Taihoku: Hinokiyama, Shinten, Sozan).

Host plant recorded on the label: *Lindera* sp. (Lauraceae); *Smilax* sp. (Smilacaceae).

Note: Syntypes, female (collected from Taiwan: Shinten, on *Smilax* sp., I-1927, by R. Takahashi (#29-2-2-2 ~29-2-3-4)).

Fiorinia taiwana Takahashi, 1934 (Fig. 3B)

(Takahashi 1934; Borchsenius 1966; Tao 1989; García Morales *et al*. 2016; Liu *et al*. 2020)

Original spelling on the label: *Fiorinia taiwana* Takah. (17 slides)

Location data on the slides: Japan (Loochoo: Naze); Taiwan (Nantou: Habon; Taihoku: Shirin, Sozan; Taito: Kaho, Toran; Takao).

Host plant recorded on the label: *Ficus* sp. (Moraceae); *Maesa* sp., *Myrica rubra* (Myricaceae); *Piper* sp. (Piperaceae); *Quercus* sp., *Quercus glauca* (Fagaceae); *Sageretia theezans* (Rhamnaceae).

Note: Syntypes, female (collected from Taiwan: Taihoku; Shirin; Sozan, on *Quercus glauca* and *Myrica rubra*, 11-X-1931, by R. Takahashi (#29-3-2-2~5、#29-3-3-1~5)).

Fiorinia theae Green, 1900 (Fig. 3C)

(Green 1900; Borchsenius 1966; Tao 1989; García Morales *et al*. 2016; Liu *et al*. 2020)

Original spelling on the label: *Fiorinia theae* Green. (11 slides)

Location data on the slides: China (Shanghai); Hozan; Taiwan (Giran: Taihezan; Heito: Mutosan; Nantou: Musha; Taihoku: Hinokiyama; Tainan: Mato).

Host plant recorded on the label: *Citrus* sp. (Rutaceae); *Eurya* sp., *Thea* sp. (Theaceae).

Fiorinia turpiniae Takahashi, 1934 (Fig. 3D)

(Takahashi 1934; Ferris 1950; Borchsenius 1966; Tao 1989, 1999; García Morales *et al*. 2016; Liu *et al*. 2020)

Original spelling on the label: *Fiorinia turpiniae* Takah. (2 slides)

Location data on the slides: Taiwan (Taichu: Kahodai).

Host plant recorded on the label: *Turpinia formosana* (Staphyleaceae).

Syn.: *Fiorinia theae turpiniae* Takahashi, 1934.

Note: Syntypes, female (collected from Taiwan: Kahodai near Hassenzan, on *Turpinia formosana*, 06-VI-1933, by R. Takahashi (#29-5-1-1~2)).

Thysanofiorinia nephelii (Maskell, 1897) (Fig. 3E)

(Maskell 1897a; MacGillivray 1921; Balachowsky 1954; Tao 1989, 1999; Wong *et al*. 1999; García Morales *et al*. 2016)

Original spelling on the label: *Fiorinia nephelii* Mask. (8 slides)

Location data on the slides: Taiwan (Kagi; Taihoku: Maruyama); Thailand (Bangkok).

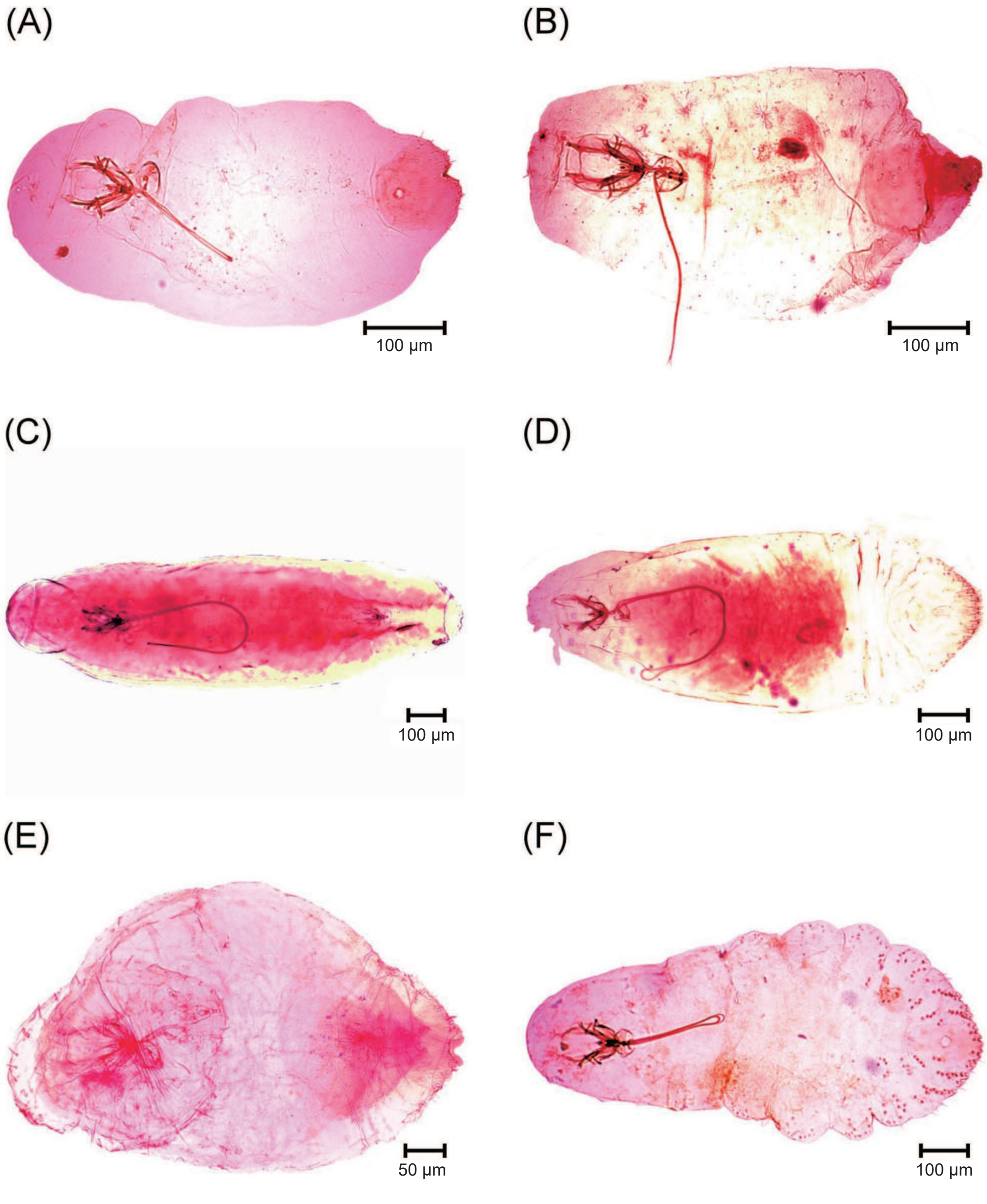

Fig. 3. (A) *Fiorinia smilaceti* Takahashi, 1931; (B) *Fiorinia taiwana* Takahashi, 1934; (C) *Fiorinia theae* Green, 1900; (D) *Fiorinia turpiniae* Takahashi, 1934; (E) *Thysanofiorinia nephelii* (Maskell, 1897); and (F) *Unachionaspis bambusae* (Cockerell, 1896).

Host plant recorded on the label: *Euphoria longana*, *Dimocarpus longan* (Sapindaceae); *Annona* sp. (Annonaceae).

Syn.: *Fiorinia nephelii* Maskell, 1897; *Fiorinia hirsuta* Marchal, 1906; *Parafiorinia hirsuta* (Marchal, 1906); *Parafiorinia nephelii* (Maskell, 1897); *Fiorinia* (*Adiscofiorinia*) *nephelii* (Maskell, 1897).

Unachionaspis bambusae (Cockerell, 1896) (Fig. 3F) (Cockerell 1896; Takagi 1961; Borchsenius 1966; Tao 1999; García Morales *et al*. 2016)

Original spelling on the label: *Chionaspis bambusae* Ckll. (1 slide)

Location data on the slides: Japan.

Host plant recorded on the label: *Bambusa* sp. (Poaceae).

Syn.: *Chionaspis bambusae* Cockerell, 1896.

Unachionaspis signata (Maskell, 1897) (Fig. 4A) (Maskell 1897a; Kuwana 1902; MacGillivray 1921; Borchsenius 1966; García Morales *et al*. 2016)

Original spelling on the label: *Chionaspis signata* Maskell. (2 slides)

Location data on the slides: Japan (Kyoto).

Host plant recorded on the label: *Bambusa* sp. (Poaceae).

Syn.: *Fiorinia signata* Maskell, 1897; *Chionaspis colemani* Kuwana, 1902; *Chionaspis signata* (Maskell, 1897); *Unachionaspis colemani* (Kuwana, 1902).

Unachionaspis tenuis (Maskell, 1897) (Fig. 4B) (Maskell 1897a; Takahashi 1936; Takahashi & Tachikawa 1956; Borchsenius 1966; García Morales *et al*. 2016)

Original spelling on the label: *Chionaspis sakaii* Takah. (3 slides)

Location data on the slides: Japan (Kyushu: Nishino-Omote Machi, Tanegashima).

Host plant recorded on the label: *Bambusa* sp. (Poaceae).

Syn.: *Fiorinia tenuis* Maskell, 1897; *Kuwanaspis tenuis* (Maskell, 1897); *Chionaspis sakaii* Takahashi, 1936.

DISCUSSION

Based on the modern taxonomic system, 20 Fioriniinae species have been collected and deposited at the TARI Insect Collection. The present paper could be a database to set up the information on scientific names, synonyms, host plants, distribution, and digital archives which will meet the requirements of both basic and applied works and will be helpful to tax-

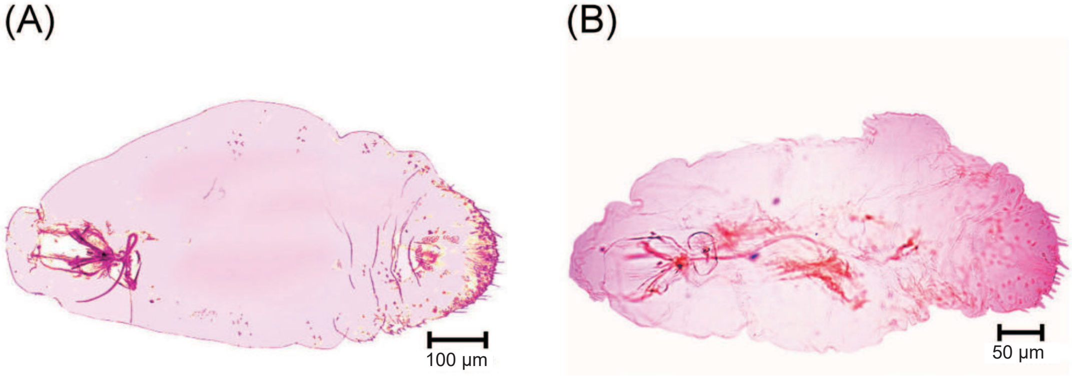

Fig. 4. (A) *Unachionaspis signata* (Maskell, 1897); and (B) *Unachionaspis tenuis* (Maskell, 1897).

onomic researchers, quarantine inspectors and farmers in the future.

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農業試驗所昆蟲標本館 (4)- 圍盾介殼蟲亞科 (半翅目：介殼蟲總科：盾介殼蟲科) 標本名錄

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摘要

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本文羅列 3 屬 20 種 (145 片玻片標本) 農業試驗所館藏之圍盾介殼蟲亞科 (Fioriniinae) [半翅目 (Hemiptera)：介殼蟲總科 (Coccoidea)：盾介殼蟲科 (Diaspididae)] 名錄，其中亦包含異名、寄主植物、採集資料及標本形態圖等資料。

關鍵詞：圍盾介殼蟲亞科、高橋良一、台灣。

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研究報告

次氯酸水對蝴蝶蘭軟腐病防治效果評估

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摘要

黃巧雯、林宗俊、曾淑瓊、賴思倫、謝廷芳、蔡佳欣。2025。次氯酸水對蝴蝶蘭軟腐病防治效果評估。台灣農業研究 74(1):37–45。

蝴蝶蘭為臺灣重要外銷花卉，年產值將近 46 億元。然而蝴蝶蘭細菌性軟腐病 (bacterial soft rot) 是影響臺灣蝴蝶蘭栽培期與外銷產值的重要病害之一。本研究利用次氯酸水 (hypochlorous acid; HOCl) 溶液作為蝴蝶蘭軟腐病防治資材，進行防治效果試驗評估。首先利用不同接種濃度的軟腐病菌測試對蝴蝶蘭軟腐病發生的影響，由結果得知，高濃度菌液 10^8 CFU mL^{-1} 接種後，其發病率為 58.3−81.3%，10^6 CFU mL^{-1} 之發病率 25.0−43.8%，但低濃度 10^4 CFU mL^{-1} 其發病率降至為 0，隨著軟腐病菌接種濃度降低，蝴蝶蘭葉片感染軟腐病之發病率逐漸下降。室內試驗結果所示，以 HOCl 溶液 10 mg L^{-1} 處理軟腐病菌 (10^8 CFU mg L^{-1}) 10 min 可完全抑制供試菌株之生長。盆栽試驗 (接種濃度 10^8 CFU mL^{-1}) 結果得知，20 mg L^{-1} HOCl 濃度下，對蝴蝶蘭軟腐病具有 18.0−66.7% 防治率，當濃度提高至 50 mg L^{-1} 下，防治率則可達 50.0−88.9%，與對照組 56.3−88.7% 之發病率有顯著性差異，顯示 HOCl 溶液可降低軟腐病的發病率。因此，本研究之結果得知，HOCl 溶液可作為田間防治蝴蝶蘭軟腐病之參考。

關鍵詞：蝴蝶蘭、軟腐病、次氯酸水、防治。

前言

蝴蝶蘭為蘭科 (Orchidaceae)、蝴蝶蘭屬 (*Phalaenopsis*) 著生性植物，原生於熱帶亞洲與太平洋上的幾個大島嶼，東至巴布亞新幾內亞，西至南印度與斯里蘭卡，南至澳洲，北至臺灣與中國雲南。臺灣的天然氣候條件，適宜多數蝴蝶蘭生長。現今臺灣蝴蝶蘭產業在產官學的共同努力發展下，在品種選育、種苗栽培管理與技術及貯運技術等多面向的精進，已使蝴蝶蘭成為臺灣之高產值與具競爭力的外銷農產品之一。根據農業部農業統計資料 (https://agrstat.moa.gov.tw/sdweb/public/maintenance/Announce.aspx)，於 2023 年蝴蝶蘭外銷出口產值高達 1.5 億美元 (約臺幣 46 億元)，主要以美國 (占 40.9%) 為最大宗的外銷國家，其次日本 (占 25.6%)、越南 (10.5%)、加拿大 (5.8%)、澳洲 (4.7%) 及印尼 (1.9%) 等國。

蝴蝶蘭於栽培期間常受到數種病害危害，根據《臺灣植物病害名彙》第五版 (Tzean *et al*. 2019) 紀錄，臺灣蝴蝶蘭主要病害有由蝴蝶蘭黃化斑點病毒 (*Phalaenopsis* chlorotic spot virus; PhCSV)、番椒黃化病毒 (*Capsicum* chlorosis virus; CaCV)、康乃馨斑駁病毒 (*Carnation* mottle virus; CarMV)、胡瓜嵌紋病毒 (*Cucumber* mosaic virus, CMV)、蕙蘭嵌紋病毒 (*Cymbidium* mosaic virus; CymMV) 及齒舌蘭輪斑病毒 (*Odontoglossum* rigspot virus; ORSV) 引起之病毒病；真菌性病害有灰黴病 (*Botrytis ci-*

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nerea)、炭疽病 (*Colletotrichum gloeosporioides*)、淡色炭疽病 (*Cylindrosporium phalaenopsidis*)、灰斑病 (*Fusarium oxysporum*)、黑斑病 (*Fusarium proliferatum*)、黃葉病 (*Fusarium solani*)、葉斑病 (*Sphaerulina phalaenopsidis*) 及疫病 (*Phytophthora palmivora*、*P. citrophthora* 及 *P. nicotianae*)；細菌性病害有褐斑病 (*Acidovorax avenae* subsp. *cattleyae*)、葉斑病 (*Burkholderia gladioli*) 及軟腐病 (*Dickeya fangzhongdai* (syn. *Pectobacterium chrysanthemi*)) (Tzean *et al*. 2019; Wei *et al*. 2021; Kao *et al*. 2023)。其中，由 *D. fangzhongdai* 引起的蝴蝶蘭細菌性軟腐病則為臺灣蝴蝶蘭栽培與外銷損耗的重要病害之一 (Wei *et al*. 2021; Lin 2022)，此病菌可感染各齡期的蝴蝶蘭葉片，花梗、花瓣皆可受害，葉片感染後初期呈現水浸狀斑，透光觀察呈半透明狀，主因為病菌藉由分泌多種細胞壁分解酵素，如果膠酶 (pectinase)、纖維素酶 (cellulase) 及蛋白酶 (protease) 等，造成植物細胞與組織崩解 (Joko *et al*. 2014)，受感染組織因而產生軟腐病徵，葉子內部組織呈現浸軟現象，但仍被葉片之角質層包裹著 (Alič *et al*. 2017)，該病斑以手輕捏病斑組織容易破裂，被害葉片組織失去支撐力下垂，3−5 d 即可造成葉片整葉腐爛，感染後期整株腐爛死亡，對於蝴蝶蘭的品質與商品價值造成嚴重的影響 (Lin 2022)。

臺灣蝴蝶蘭軟腐病的主要防治方法除了藉由罹病株立即移除的清園工作、避免密植減少葉片摩擦造成傷口、加強園區內通風及降低園區內濕度來減少病原菌增殖外，目前國內尚無核准使用之推薦藥劑提供防治蝴蝶蘭軟腐病。此外，臺灣的蝴蝶蘭在外銷檢疫規定中，部分國家要求嚴格不得使用抗生素進行細菌性病害管理。然而，近年來全世界 COVID-19 疫情流行期間，除了酒精以外，次氯酸水 (hypochlorous acid; HOCl) 是很好的環境清潔消毒劑。依據 2017 年 6 月 12 日衛生福利部公告 (https://www.mohw.gov.tw/cp-3250-29989-1.html)，臺灣將 HOCl 列為食品用清潔劑，也就是可用於作為水的殺菌或是食品、容器及食材洗滌之用，在食材殘留不得超過 1 mg L^{-1}。HOCl 在美國與日本等國家亦允許應用於醫療照護、畜牧、水產業及農產品加工上 (Eryilmaz & Palabiyik 2013; Jafry *et al*. 2017)。本研究利用 HOCl 溶液作為防治資材，探討蝴蝶蘭細菌性軟腐病之防治效果評估，以作為防治本病害之參考。

材料與方法

供試藥劑、植株及菌株來源

本研究使用之 HOCl 溶液，係採用微酸性電解水生成機 (HOCl 0.2 t，衛康商貿股份有限公司，臺灣桃園市) 製備完成 HOCl 原液濃度，直接加水稀釋至測試所需的濃度即可使用。本研究使用蝴蝶蘭植株，係購買自嘉義縣大林鎮蘭園，品種為白花蝴蝶蘭 (*Phal*. Sogo Yukidian ‘V3’)。*Dickeya fangzhongdai* Di-001、Di-002 菌株 (簡稱 Di-001、Di-002) 係農業試驗所細菌研究室保存之菌株。

不同接種濃度的軟腐病菌對蝴蝶蘭軟腐病發生之影響

首先將測試之 Di-001 菌株培養於營養培養基 Nutrient Agar (NA; BD Difco™, Franklin-Lakes, NJ, USA) 培養基，於 25℃培養 24–48 h 後懸浮於無菌水，再利用分光光度計 (spectrophotometer, Spectronic 70, Bausch & Lomb., Bridgewater, NJ, USA) 於波長 600 nm 下，調整吸收值 OD (optical density) 至 0.3，此濃度約含 10^8 CFU mL^{-1}，接著以無菌水進行 10 倍稀釋，調整接種濃度。以滅過菌蟲針在葉片上製造傷口，取 20 μL 軟腐病菌接種濃度滴於傷口上，每片葉片於左右兩側各處理一點，每盆接種 2 片葉片，每處理 4 盆，並以無菌水作為對照組，之後放置於 28℃之定溫箱內並逐日觀察病斑進展情形與記錄，本試驗觀察至 5 d 止，本實驗重複進行 2 次。軟腐病之發病率計算公式如下：發病率 (disease incidence, %) = (植株葉片上出現軟腐病徵之罹病接種數/調查總接種數) × 100%。

HOCl 溶液對蝴蝶蘭軟腐病菌之殺菌效率

將測試之 Di-001 與 Di-002 兩菌株培養於 NA 培養基，於 25℃ 培養 24−48 h 後懸浮於無菌水，配製成 10^8 CFU mL^{-1} 的細胞懸浮液 (OD_{600} = 0.3)。將原液濃度為 200 mg L^{-1} 之 HOCl 溶液以 0.22 um Millipore 過濾膜 (Millex® syringe filters, Millipore, Burlington, MA, USA) 過濾滅菌後，配製濃度分別為 2、10、20、40、100 及 200 mg L^{-1} 之 HOCl 溶液。於 1.5 mL 滅菌保存管中分別加入各濃度之 HOCl 溶液 200 µL 與測試菌株懸浮液 200 µL，使其 HOCl 最終濃度分別為 1、5、10、20、50 及 100 mg L^{-1}，另外細菌懸浮液添加無菌水之處理作為對照。利用震盪培養於 150 rpm 下，震盪處理約 10 min，隨即置入離心機 (Heraeus Megafuge 8 Centrifuge, Thermo Scientific, Germany) 以 3,000 rpm 離心 2 min，將細菌與藥劑分離，倒掉上層液並加入 1 mL 無菌水懸浮後，以微量吸管吸取 50 µL 懸浮液塗抹於 NA 培養基，每個處理 4 重複，於 25℃ 培養 24−48 h，觀察細菌是否生長而形成菌落，並以 “+” 表示細菌菌落生長；“–” 表示無細菌菌落生長。

HOCl 溶液防治蝴蝶蘭軟腐病之盆栽試驗

如上述製備濃度約為 10^8 CFU mL^{-1} 之 Di-001 菌株懸浮液。供試植株如上述，購買後至回溫室生長至少 4 wk，用蟲針於蝴蝶蘭葉片上製造傷口後進行接種處理，再以滅菌之微量吸管吸取 20 µL 細胞懸浮液 (10^8 CFU mL^{-1})，接種後 30 min 以前述配製濃度分別為 5、10、20、50 及 100 mg L^{-1} 之 HOCl 溶液進行噴霧方式於接種處，另以無菌水作為對照組，每個處理 4 盆，之後放置於溫室，每天觀察並記錄病害發生情形，本試驗觀察至 5 d 止，以評估其防治效果，本試驗重複進行 2 次。

統計分析

各項處理之試驗資料利用統計分析軟體 SAS Enterprise Guide 7.1 版先進行變方分析 (analysis of variance; ANOVA)，再以最小顯著性差異 (least significant difference; LSD; P = 0.05) 測驗，在 5% 顯著水準下比較處理間平均值之差異。

結果

不同接種濃度的軟腐病菌對蝴蝶蘭軟腐病發生之影響

結果如表 1 所示，第一次試驗結果顯示，接種高濃度 10^8 與 10^7 CFU mL^{-1} Di-001 細胞懸浮液，於接種後第 1 天即開始出現病徵，接種點出現水浸狀病斑，於接種後第 2 天軟腐病徵擴大造成整片葉片軟爛、下垂現象，其發病率分別為 58.3% 與 33.3%；接種濃度為 10^6 CFU mL^{-1} Di-001 細胞懸浮液，於接種後第 3 天開始出現水浸狀病斑，之後病斑逐漸擴大，其發病率為 25.0%；接種較低濃度 10^5 CFU mL^{-1} Di-001 細胞懸浮液，則於接種後第 5 天出現病徵，但病勢進展呈現停滯狀態，其發病率為 8.3%；接種濃度降至 10^4 CFU mL^{-1} Di-001 細胞懸浮液，則於接種後 7 d 未見病徵

表 1. 不同接種濃度 (colony-forming unit (CFU) mL^{-1}) 之軟腐病菌對蝴蝶蘭軟腐病發生之影響。

Table 1. Effect of different inoculum concentrations (colony-forming unit (CFU) mL^{-1}) of *Dickeya fangzhongdai* Di-001 on the occurrence of Phalaenopsis soft rot disease.

Colony-forming unit (CFU) mL^{-1}	Disease incidence (%)^z	
	EXP1	EXP2
CK^y	0.0 c^x	0.0 c
4	0.0 c	0.0 c
5	8.3 bc	12.5 c
6	25.0 bc	43.8 b
7	33.3 ab	62.5 ab
8	58.3 a	81.3 a
LSD	31.4	26.9

^{z} Incidence (%) = (Number of *Phalaenopsis* showed symptoms of soft rot/Total number of inoculated *Phalaenopsis*) × 100%.
^{y} Inoculation with sterile water as control.
^{x} Means within a column followed by the same letter are not significantly different at 5% by least significant difference (LSD) test.

表現，對照組以無菌水處理其發病率為 0。而在第二次試驗中，接種高濃度 10^8 與 10^7 CFU mL^{-1} Di-001 細胞懸浮液，其發病率分別為 81.3% 與 62.5%；接種濃度為 10^6 CFU mL^{-1} Di-001 細胞懸浮液，其發病率為 43.8%；當接種濃度降至為 10^5 CFU mL^{-1} Di-001 細胞懸浮液，發病率則為 12.5%；當接種濃度於 10^4 CFU mL^{-1} Di-001 細胞懸浮液，則於植株葉片上未見病徵表現。

HOCl 溶液對蝴蝶蘭軟腐病菌之殺菌效率

供試 Di-001 與 Di-002 菌株以 10 mg L^{-1} 以上之 HOCl 溶液震盪處理 10 min 後，Di-001 菌株於 NA 培養基上可見 2–4 間零星菌落數量 (圖 1)，而 Di-002 菌株生長則被完全抑制，當 HOCl 溶液濃度提高 20 mg L^{-1}，供試兩菌株之菌落則被完全抑制 (表 2)。以 Di-001 菌株測試時，1 mg L^{-1} HOCl 溶液處理 10 min 即可將該菌株之族群量由原本 3.36×10^8 CFU mL^{-1} 降低至 0.83×10^8 CFU mL^{-1}，其該菌株之族群量降低 2.53 個 log 值，而在 20 mg L^{-1} 以上 HOCl 處理 10 min 後，則無活菌的存在 (表 3、圖 1)。

表 2. HOCl 溶液對蝴蝶蘭軟腐病菌不同菌株之殺菌效果。

Table 2. Bactericidal efficacy of hypochlorous acid (HOCl) solution against strains of *Dickeya fangzhongdai* Di-001 and Di-002.

	D. fangzhongdai[z]	
HOCl (mg L^{-1})	Di-001	Di-002
0	+[y]	+
1	+	+
5	+	+
10	+	–
20	–	–
50	–	–
100	–	–

[z] Two hundred μL of different concentrations of HOCl solution was mixed with 200 μL of 10^8 CFU mL^{-1} of Di suspension. The final concentrations of HOCl in the mixtures were 1, 5, 10, 20, 50, and 100 mg L^{-1}. After mixing and shaking for 10 min, the bacterial cells were separated by centrifugation and suspended in sterile distilled water. The suspension was serially diluted and plated on agar plates for counting the survived bacterial cells.

[y] "+" indicates bacterial growth and "–" indicates no bacterial growth.

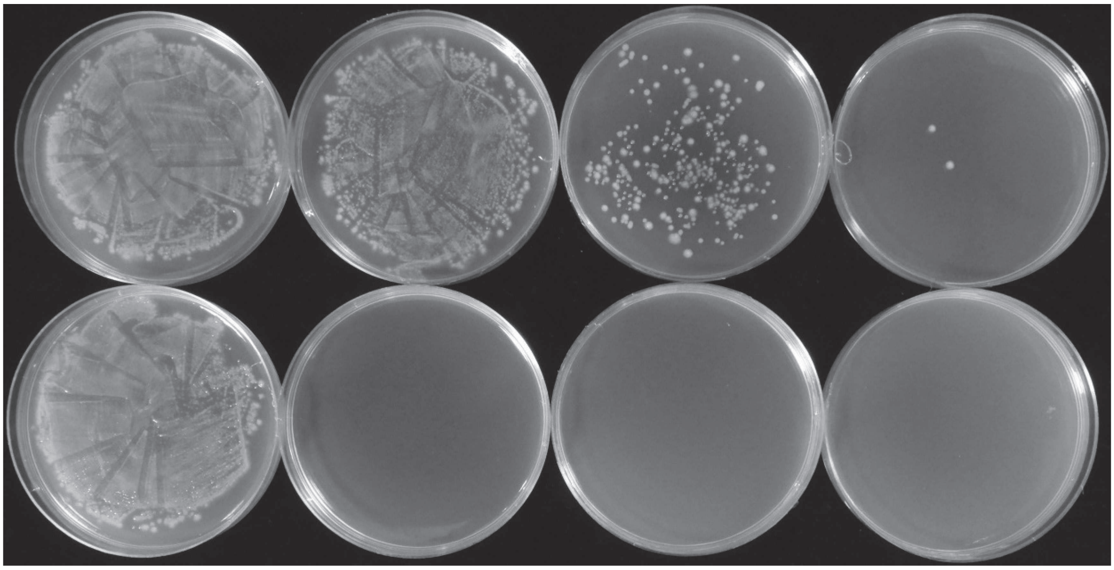

圖 1. HOCl 溶液對蝴蝶蘭軟腐病菌菌落 10^8 CFU mL^{-1} 之影響。由左到右，HOCl 濃度分別為 0、1、5、10 (上) 與 0、20、50、100 (下) mg L^{-1}。

Fig. 1. Effect of different concentration of hypochlorous acid (HOCl) solution against strains of *Dickeya fangzhongdai* Di-001. From left to right, the concentrations of HOCl are 0, 1, 5, 10 (up) and 0, 20, 50, 100 (under) mg L^{-1}.

表 3. 軟腐病菌 Di-001 菌株經 HOCl 溶液處理 10 min 之族群減少量。

Table 3. Reducion of populations of *Dickeya fangzhongdai* Di-001 in water after treatment with different concentrations of hypochlorous acid solution for 10 min.

HOCl (mg L^{-1})	Population (log CFU mL^{-1})^z Before treatment	After treatment	Log reduction after HOCl treatment (log CFU mL^{-1})^y
0	3.36	3.36	0.00
1	3.36	0.83	2.53
5	3.36	0.03	3.33
10	3.36	≃ 0.00	≃ 3.36
20	3.36	N.D^x	≥ 3.36
50	3.36	N.D	≥ 3.36
100	3.36	N.D	≥ 3.36

^{z} Two hundred μL of different concentrations of HOCl solution was mixed with 200 μL of 10^8 CFU mL^{-1} of Di-001 suspension. The final concentrations of HOCl in the mixtures were 1, 5, 10, 20, 50, and 100 mg L^{-1}. After mixing and shaking for 10 min, the bacterial cells were separated by centrifugation and suspended in sterile distilled water. The suspension was serially diluted and plated on agar plates for counting the survived bacterial cells.

^{y} Log reduction = population after mixing and shaking for 10 min with sterile distilled water-population after HOCl solution treatment.

^{x} N.D: viable *D. fangzhongdai* Di-001 was not detected by the dilution plate method.

HOCl 溶液防治蝴蝶蘭軟腐病之盆栽試驗

結果如表 4 所示，第一次試驗結果顯示，HOCl 溶液 20、50 及 100 mg L^{-1} 處理組之蝴蝶蘭軟腐病發病率分別為 18.8%、6.3% 及 0.0%，與對照組 56.3% 之發病率有顯著性差異 (P = 0.05)；而在第二次試驗中，HOCl 溶液 50 mg L^{-1} 與 100 mg L^{-1} 處理組之蝴蝶蘭軟腐病發病率分別為 44.3% 與 22.0%，亦與對照組 88.7% 之發病率有顯著性差異 (P = 0.05) (圖 2)。

討論

蝴蝶蘭細菌性軟腐病為臺灣蝴蝶蘭產業重要的病害之一，在臺灣蘭園普遍發生，嚴重影響蝴蝶蘭的品質與商品價值 (Wei *et al*. 2021; Lin 2022)。根據前人研究調查資料得知，細菌性軟腐病菌普遍發生於全球各地，其寄主範圍廣泛，涵蓋多種重要蔬菜與花卉作物如甘藷、

表 4. HOCl 溶液對蝴蝶蘭軟腐病發生之影響。

Table 4. Effect of hypochlorous acid (HOCl) solution treatment on incidence of *Phalaenopsis* soft rot disease caused by *Dickeya fangzhongdai*.

HOCl (mg L^{-1})	Disease incidence (%)^z EXP1	EXP2
0	56.3 a^y	88.7 a
5	50.0 a	87.0 a
10	43.8 ab	77.7 a
20	18.8 bc	72.0 a
50	6.3 c	44.3 b
100	0.0 c	22.0 b
LSD	29.0	27.2

^{z} Disease incidence (%) = (Number of leaves which showed symptoms of soft rot/Total number of inoculated leaves) × 100%.

^{y} Means within a column followed by the same letter are not significantly different at 5% by least significant difference (LSD) test.

馬鈴薯、芹菜、蔥、牛蒡、玉米、胡蘿蔔、胡瓜、番椒、十字花科作物、洋蔥、康乃馨、百合花、非洲菫、牡丹花、海芋、蘭科花卉 (蝴蝶蘭、文心蘭、石斛蘭、拖鞋蘭) 及菊花等造成嚴重的經濟損失 (Ma *et al*. 2007; Joko *et al*. 2014; Van Gijsegem *et al*. 2021)。目前從臺灣的蝴蝶蘭所分離到的軟腐細菌，經由多位點序列分析 (multilocus sequence analysis) 鑑定後，皆屬於 *D. fangzhongdai* (Wei *et al*. 2021; Lin 2022)。蝴蝶蘭軟腐病菌在高溫高濕 (28℃，相對溼度大於 80%) 的環境條件合適本菌侵染危害植株 (Toth *et al*. 2003, 2021; Mcmillan *et al*. 2007)，在溫室栽培的蝴蝶蘭，由於室內溫度高且採淋灌或噴灌方式，適合軟腐病發生的環境條件，本病害除冬天較少見外，其他季節均可見其發生，為普遍且嚴重的病害之一。軟腐細菌為革蘭式陰性菌 (gram-negative bacteria) 與兼性厭氧菌 (facultative anaerobic bacteria)，且為一種伺機性病原菌 (opportunistic pathogens)，主要透過傷口或自然開口侵入植物組織，本研究在葉片製造傷口後，測試不同接種濃度的軟腐病菌對蝴蝶蘭軟腐病發生的影響，由結果得知，高濃度 10^8 CFU mL^{-1} 其發病率高達 58.3−81.3%，10^6 CFU mL^{-1} 仍有 25.0−43.8% 發病率，但低濃度 10^4 CFU mL^{-1} 其發病

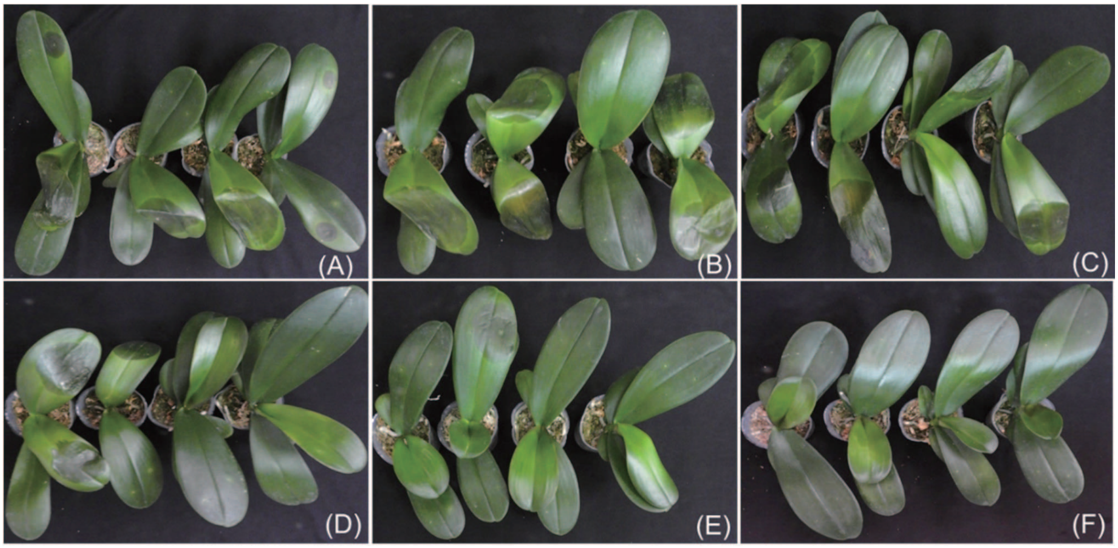

圖 2.　HOCl 溶液對蝴蝶蘭軟腐病發生之防治評估。(A) 蝴蝶蘭接種軟腐病菌後，以水處理。(B–F) 蝴蝶蘭接種軟腐病菌後，依序以 HOCl 溶液 5、10、20、50 及 100 mg L^{-1} 處理。

Fig. 2.　Control evaluation of *Dickeya fangzhongdai* Di-001 by spraying treatment with hypochlorous acid (HOCl) solution (B) 5 mg L^{-1}; (C) 10 mg L^{-1}; (D) 20 mg L^{-1}; (E) 50 mg L^{-1}; (F) 100 mg L^{-1}; and (A) sterile distilled water for 5 d at 28℃ .

率為 0，隨著軟腐病菌接種濃度越低，蝴蝶蘭葉片感染軟腐病之發病率逐漸降低。值得注意的是，當植株上出現病徵時，推測其病原菌密度至少有 10^5 CFU mL^{-1} 濃度，建議本病害防治仍應將出現病徵的植株並帶離園區，以降低田間感染源密度。

有鑑於目前國內尚無核准使用之推薦藥劑提供業者防治蝴蝶蘭軟腐病，又臺灣的蝴蝶蘭在外銷檢疫規定中，部分國家要求嚴格不得使用抗生素進行細菌性病害管理。本研究利用 HOCl 溶液作為防治資材，評估此防治資材用於蝴蝶蘭軟腐病之防治效果。本研究發現 HOCl 溶液在濃度 10 mg L^{-1} 下對蝴蝶蘭軟腐病菌之殺菌效率，由 NA 培養皿試驗得知菌落數介於 0–4 間極稀少數量，若 HOCl 濃度提高至 20 mg L^{-1} 下則無活菌的存在，顯示隨著 HOCl 溶液濃度增加，對蝴蝶蘭軟腐病菌之殺菌效率隨之增加；盆栽試驗結果得知，20 mg L^{-1} HOCl 濃度下，對蝴蝶蘭軟腐病具有 18.8−66.7% 防治率，當濃度提高至 50 mg L^{-1} 下，防治率則可達 50.0−88.9%，顯示 HOCl 溶液處理其蝴蝶蘭軟腐病發生率均顯著低於對照未處理之發生率。目前已有許多研究報導證實 HOCl 溶液可使細菌、病毒、真菌及原生動物失去活性 (Thorn *et al*. 2012; Sakarya *et al*. 2014; Hughson *et al*. 2016)，亦可應用於蝴蝶蘭軟腐病之防治，但使用上應須注意 HOCl 之濃度。為瞭解 HOCl 施用於蝴蝶蘭植株上是否有藥害風險問題，本研究於白花蝴蝶蘭 (*Phal*. Sogo Yukidian ‘V3’) 品種上進行 HOCl 100 mg L^{-1} 之施用，於成熟葉與嫩葉葉片上皆未出現疑似藥害病斑 (數據未顯示)，但因蝴蝶蘭品系眾多，建議使用前須先行小區域施測試，應注意是否有藥害發生。本研究中所測試的 HOCl 為氯系的氧化劑，因具強氧化性，造成蛋白質或 DNA 的變性，並破壞微生物細胞膜通透性達到殺菌的效果 (Saikumar *et al*. 2023)。美國環境保護署 (U.S. Environmental Protection Agency; EPA) 批准 HOCl 可針對 SARS-CoV-2 的消毒劑以對抗 COVID-19，美國食品藥物管理局 (U.S. Food and Drug Administration; FDA) 亦也批准可用於食品加工設備、水果及蔬菜的表面消毒 (Okamoto *et al*. 2020; Saikumar *et al*. 2023)。在臺灣，依據

2017 年 6 月 12 日衛生福利部公告，將 HOCl 列為食品用清潔劑，可用於作為水的殺菌或是食品、容器及食材洗滌之用。由於 HOCl 溶液之 pH 值偏弱酸性，刺激性較低，對環境無害、無毒性等優點，但遇光則分解，高光照強度與紫外線易使，HOCl 產生降解作用 (Ishihara *et al*. 2017) 等缺點，HOCl 50 ppm 濃度以密封遮光容器裝，存放於陰涼處，2 mo 後其濃度變化不大，但若保存不當，其濃度降解速度快，可能於 1 wk 內無殺菌效果；HOCl 使用於通風良好環境，避免使用在金屬材質上。然而，近年來全世界 COVID-19 疫情流行期間，除了酒精以外，HOCl 是很好的環境清潔消毒劑。

由於軟腐病菌主要藉傷口或自然開口侵入組織，經由葉面澆水、噴霧造成葉片上游離水有利於病菌的侵染而散播至其他健康植株 (Su & Leu 1992)，擴散快速，病害發生後無法根治，易造成嚴重損失 (Joko *et al*. 2014; Toth *et al*. 2021)。加強田間衛生仍為首要重要工作，將罹病葉、罹病植株移除以減少園區內感染密度。另外，調整栽植密度，改善園內環境通風性，減少葉片上游離水停留的時間，亦能降低病害發生。適當施肥外仍需避免氮肥過度施用，維持植株生長勢良好，均是預防蝴蝶蘭軟腐病發生的基本防治策略。最後，適當使用防治資材以進行早期保護，避免對蝴蝶蘭產量及品質產生嚴重損失，影響業者收益。本研究已證實 HOCl 溶液對軟腐病之防治效果佳，建議濃度為 20–50 mg L^{-1} 來防治蝴蝶蘭軟腐病，平時可以 10 mg L^{-1} HOCl 溶液進行園區例行管理，降低園區內病原菌密度。HOCl 溶液為應用範圍廣且效果佳之植物病害防治資材，對環境衝擊低，亦為免登記植物保護資材 (次氯酸鹽類，Hypochlorites)，以上資訊可供業者防治蝴蝶蘭軟腐病之參考。

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Evaluation of Control Efficacy of Hypochlorous Acid Against *Phalaenopsis* Soft Rot

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Abstract

Huang, C. W., T. C. Lin, S. J. Zeng, S. L. Lai, T. F. Hsieh, and C. H. Tsai. 2025. Evaluation of control efficacy of hypochlorous acid against *Phalaenopsis* soft rot. J. Taiwan Agric. Res. 74(1):37–45.

Phalaenopsis orchids are important export flowers in Taiwan, with an annual production value of nearly 4.6 billion NT dollars. However, bacterial soft rot significantly affects their cultivation and export value. Therefore, this study evaluates the effectiveness of hypochlorous acid (HOCl) solution as a control material for preventing bacterial soft rot in *Phalaenopsis* orchids. Different inoculum concentrations of the rot bacteria were tested to assess their impact on disease occurrence. Results showed that at a high concentration of 10^8 CFU mL^{-1}, the disease incidence was as high as 58.3−81.3%, while at 10^6 CFU mL^{-1}, it was 25.0−43.8%. At a low concentration of 10^4 CFU mL^{-1}, the incidence decreased to 0%, indicating a gradual reduction in disease occurrence with lower bacterial concentrations. Laboratory experiments demonstrated that treating the bacteria (10^8 CFU mL^{-1}) with a 10 mg L^{-1} HOCl solution for 10 min inhibited their growth. Pot experiments revealed that at a HOCl concentration of 20 mg L^{-1}, the control efficacy against bacterial soft rot ranged from 18.0% to 66.7%. In contrast, the control efficacy increased from 50.0% to 88.9% at 50 mg L^{-1}, showing significant differences in control effectiveness. Thus, HOCl solution can control the bacterial disease of *Phalaenopsis* soft rot caused by *Dickeya fangzhongdai* in orchids.

Key words: *Phalaenopsis*, Hypochlorous acid, Soft rot, *Dickeya fangzhongdai*, control.

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Copula 函數在水稻產量及價格相依性分析之研究

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摘要

廖大經。2025。Copula 函數在水稻產量及價格相依性分析之研究。台灣農業研究 74(1):47–61。

本研究主要目的在於透過 Copula 函數分析嘉義縣水稻栽培主要鄉鎮市之產量與價格變數之相依性，以作為估算收入風險與稻作生產調適措施之參考。分析結果顯示，除第 1 期作義竹鄉、第 2 期作中埔鄉及六腳鄉外，其他鄉鎮市在兩期作之水稻產量與價格之 Kendall's tau 相關係數均呈負相關，然而相關係數偏低，相關性表現微弱。在尾部相依性方面，第 1 期作義竹鄉為上尾相關，第 2 期作中埔鄉、民雄鄉及新港鄉為對稱尾部相關，六腳鄉為上尾相關，其他鄉鎮市在兩期作均無尾部相關或下尾相關。但值得注意的是，第 1 期作有多數鄉鎮市產量與價格耦合配適之 Copula 函數，均屬於對下尾相關敏感的 Clayton 函數。由於近年第 1 期作水稻生育後期經常受到極端天氣影響，因此造成低產與低價同時發生下尾相關的潛在風險仍不可忽視。最後模擬各鄉鎮市水稻收入期望損失以評估收入風險，發現除民雄鄉外，其他各鄉鎮市第 2 期作的期望損失均高於第 1 期作，可能與第 2 期作生育初期氣象災害，以及後期日射量較低有關，造成稻作生產風險較高。比較各鄉鎮市期望損失高低，兩期作均以六腳鄉與義竹鄉較高，推測係因沿海地區稻作栽培環境較為嚴苛，收入波動幅度較大所致。未來可透過開發栽培調適技術或選育耐逆境新品種，藉此提高沿海地區稻作生產韌性，以降低收入風險。

關鍵詞：水稻、Copula 函數、相依性。

前言

水稻是臺灣農業栽培面積最大之單一作物。據統計，2022 年臺灣水稻全年收穫面積為 238,701 ha (https://agrstat.moa.gov.tw/sdweb/public/book/Book.aspx)。以稻作生產為核心所形成之稻作產業鏈，更是維持農業經濟與糧食安全穩定的重要支柱。近年由於氣候變遷的影響，極端天氣事件對於作物生產之致災頻率與強度提高，因此造成產量與收益的風險均較過去增加。

水稻收入主要由產量與價格 2 項因素所決定，因此必須同時評估產量與價格的風險。因此，如何正確推估產量與價格的邊際機率及聯合機率分布，對於合理評估收入風險將有相當大的助益。在理想狀況下，一般均假設產量與價格其聯合機率分布為二元常態分布，亦即變數之間呈 Gaussian 對稱相關結構。然而，實際上該假設未必合適，因為產量與價格存在相依性 (dependence) (Embrechts *et al*. 2002)。變數間的相關性 (correlation)，一般泛指線性相關的程度，通常只是相依性的一種特殊情況，例如產量與價格的關係一般呈負相關，因此具有風險對沖的效果 (Tejeda & Goodwin 2008)。但是水稻基於歷史與糧食安全的特殊性，其價格可能受到政策調節的影響，並非完全由市場機制所決定，故而產量與價格的相依關係可能除簡單的線性相關之外，更為複雜多變，也使得欲正確配適其聯合機率分布的數學推導過程將極為困難。

Sklar (1959) 提出一種可耦合多個相依變數邊際機率與聯合機率分布的函數，是一個

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簡化配適多元聯合機率分布複雜度的有力工具，稱為 Copula 函數。早年受到計算工具能力所限，Copula 函數未受到太多注目。近年隨著電腦算力與效率的大幅提升，Copula 函數藉著無需事前假設變數之邊際機率分布、可任意結合不同邊際機率分布之相依變數配適其聯合機率分布及尾部相依風險分析等優勢，因此 Copula 函數在金融投資避險與自然災害多維風險評估方面的應用相當廣泛。本研究以水稻為研究對象，根據不同年度、期作及地區之產量與價格統計資料，配適兩變數邊際機率分布與評估其相依性，再利用 Copula 函數配適收入聯合機率分布，並且在近似變數固有相依性的情況下，進一步以蒙地卡羅方法 (Monte Carlo method) 模擬水稻收入分布資料，據此探討水稻收入風險分布在不同期作與地區的差異，以及是否具有地緣特性，可提供作為未來相關政策規劃的學術參考。

材料與方法

統計資料

本研究分析之水稻產量與價格資料以稻穀為主，係分別引用自農糧署之『農情報告資源網』(https://agr.afa.gov.tw/afa/afa_frame.jsp) 與『糧價、糧商及當旬公糧收購數量資訊查詢系統』(https://foodinformation.afa.gov.tw/Search/S02/S0201/S02010101.aspx?sys_id=S02&sys_pid=S02010101) 網頁中，關於 2007–2022 年嘉義縣各鄉鎮市第 1、2 期作稉稻資料 (價格僅有嘉義縣資料，未及各鄉鎮市)。其中產量資料係各鄉鎮市第 1、2 期作之稉稻稻穀期作產量，價格資料第 1 期作係嘉義縣 6、7、8 月份平均值；第 2 期作係嘉義縣 10、11、12 月份平均值。

資料預處理

資料預處理有通貨膨脹指數校正與時間序列 (time series) 除趨勢化 2 步驟，分別說明如次。

稻穀價格資料校正

為消除通貨膨脹的影響，使不同年度之稻穀價格資料具可比較性，本研究使用行政院主計總處公布之躉售物價指數 (wholesale price index; WPI) 稻穀基本物價分類指數 (https://nstatdb.dgbas.gov.tw/dgbasAll/webMain.aspx?sys=100&funid=dgmaine) (以 2016 年為基期) 對於價格歷史資料進行校正。價格校正公式如式 (1) 所示：

$$P_t^{adj} = P_t^{raw}\left(\frac{W_t}{W_T}\right),\ t = 1, 2, \ldots, T \qquad (1)$$

式 (1) 中 P_t^{adj} 表示為第 t 期 (年度) 之校正後價格資料；P_t^{raw} 表示為第 t 期之原始價格資料；W_t 表示為第 t 期之 WPI；W_T 表示為最後 1 期之 WPI。

稻穀產量及價格資料除趨勢化

由於產量與價格資料屬於時間序列資料，為消除資料因外在因素影響而隨著時間推移所表現的某種趨勢，例如產量隨著生產技術的進步而逐年增產，或者價格隨著每人年均消費量減少而降低，以獲得真正表現隨機風險的資料。本研究使用 Hodrick & Prescott (1997) 提出之 HP 濾波法 (Hodrick-Prescott filter; HP) 進行資料除趨勢化工作。HP 濾波法本質上屬於帶通濾波器 (band-pass filter)，係先將時間序列資料 y_t 分解為趨勢項 (trend component) τ_t 與週期項 (cyclical component) c_t 兩種成分，即：

$$y_t = \tau_t + c_t \qquad (2)$$

接著藉由定義最小化函數 (Kim 2004)：

$$min\left(\sum_{t=1}^{T}(y_t - \tau_t)^2 + \lambda\sum_{t=2}^{T-1}\left[(\tau_{t+1} - \tau_t) - (\tau_t - \tau_{t-1})\right]^2\right) \qquad (3)$$

並利用最小平方法求得 τ_t 之最佳解。式 (3) 中包含兩項平方和，第 1 項平方和係藉由懲罰性平方和之最小化控制趨勢項 τ_t 與整體時間序列 y_t 的偏差大小，使其 τ_t 對於 y_t 為最佳配適。第 2 項平方和係藉由 τ_t 之二階差分控制 τ_t 曲線的平滑程度，λ 為趨勢平滑係數，λ 值越大則趨

勢曲線越平滑。至於設定 λ 值大小係視資料觀測頻率而定，Hodrick & Prescott (1997) 建議 λ 值設定方式如式 (4) 所示：

$$\lambda = 1600\left(\frac{f}{4}\right)^2 \tag{4}$$

式 (4) 中，f 為觀測頻率，即資料為季度資料則 $f = 4$，λ = 1,600；若為月度資料則 $f = 12$，λ = 14,400；若為年度資料則 $f = 1$，λ = 100。Ravn & Uhlig (2002) 則認為較為合理之 λ 值大小應調整為式 (5) 所示：

$$\lambda = 1600\left(\frac{f}{4}\right)^4 \tag{5}$$

亦即季度資料 λ = 1,600；年度資料 λ = 6.25；月度資料 λ = 129,600。

資料標準化 (standardization)

產量與價格資料經過去趨勢化之後，為了使資料具有可比較性，因此進行資料的標準化，將各年度資料 y_t 與最新年度資料 y_T 對齊。本研究採用 Yang *et al*. (2021) 的標準化公式：

$$y_t' = \tau_T + \left(\frac{c_t}{\tau_t}\right)\tau_T,\ t = 1, 2, \ldots, T \tag{6}$$

式 (6) 中，y_t' 為 y_t 經過標準化的資料；τ_t 與 c_t 各為 y_t 之趨勢與週期成分；τ_T 為 y_T 之趨勢成分。

水稻產量與價格相依性分析

本研究使用 Copula 函數對於水稻產量與價格之相依性進行分析，關於 Copula 函數與分析步驟說明如次。

Copula 函數

Copula 函數可簡化多元隨機變數之間相依性問題的複雜度，最早由 Sklar (1959) 以數學定理說明 Copula 函數與多元聯合分布的關係，假設有多元連續邊際分布 $F_1(x_1), F_2(x_2), \ldots, F_n(x_n)$，若其聯合分布為 $H(x_1, x_2, \ldots, x_n)$，則必定存在唯一 Copula 函數 C，使得：

$$\begin{aligned} &H(x_1, x_2, \ldots, x_n) \\ &= C\big(F_1(x_1), F_2(x_2), \ldots, F_n(x_n)\big) \end{aligned} \tag{7}$$

式 (7) 表示 Copula 函數 C 將 n 個邊際分布耦合在一起，得到一個聯合分布 H。反之，若已知多元連續邊際分布，可令隨機變數 $R_1 = F_1(x_1)$, $R_2 = F_2(x_2), \ldots, R_n = F_n(x_n)$，根據機率積分變換定理，新的隨機變數 $R_1, R_2, \ldots, R_n$ 皆服從均勻分布 $U_n(0, 1)$，如此可求得 Copula 函數 C 如式 (8) 所示：

$$\begin{aligned} &C(r_1, r_2, \ldots, r_n) \\ &= H\big(F^{-1}(r_1), F^{-1}(r_2), \ldots, F^{-1}(r_n)\big) \end{aligned} \tag{8}$$

本研究選擇 5 種 Copula 函數來耦合產量與價格變數，分別是屬於橢圓族 (elliptical copulas) 的 Gaussian copula 與 t copula，以及屬於阿基米德族 (Archimedean copulas) 的 Clayton copula、Gumbel copula 及 Frank copula。5 種 Copula 函數的數學表達式如下所示：

1. Gaussian copula

$$C_\tau^{Ga}(r_1, r_2) = \Phi_\tau\big(\Phi^{-1}(r_1), \Phi^{-1}(r_2)\big) \tag{9}$$

式 (9) 中，C_τ^{Ga} 表示 Gaussian copula 函數，Φ_τ 表示相關係數為 τ 之二維標準常態分布函數，Φ^{-1} 為標準常態分布之反函數。

2. t copula

$$C_{\nu,\tau}^{t}(r_1, r_2) = t_{\nu,\tau}\big(t_\nu^{-1}(r_1), t_\nu^{-1}(r_2)\big) \tag{10}$$

式 (10) 中，$C_{\nu,\tau}^{t}$ 表示 t copula 函數，$t_{\nu,\tau}$ 表示自由度為 ν、相關係數為 τ 之二維 Student's t 分布函數，t_ν^{-1} 表示自由度為 ν 之 Student's t 分布反函數。

3. Clayton copula (Nelsen 2006)

$$\begin{aligned} &C_\theta^{Cl}(r_1, r_2) = \big[\max(r_1^{-\theta} + r_2^{-\theta} - 1, 0)\big]^{-\frac{1}{\theta}} \\ &\theta \in [-1, \infty)\backslash\{0\} \end{aligned} \tag{11}$$

式 (11) 中，C_θ^{Cl} 表示 Clayton copula 函數，θ 為 Copula 函數之相依性參數。

4. Gumbell copula (Nelsen 2006)

$$C_\theta^{Cu}(r_1, r_2) = \exp\left(-\left[(-\ln r_1)^\theta + (-\ln r_2)^\theta\right]^{-\frac{1}{\theta}}\right) \quad (12)$$
$$\theta \in [1, \infty)$$

式 (12) 中，C_θ^{Gu} 表示 Gumbell copula 函數，θ 為 Copula 函數之相依性參數。

5. Frank copula (Nelsen 2006)

$$C_\theta^{Fr}(r_1, r_2) = -\frac{1}{\theta}\ln\left(1 + \frac{(e^{-\theta r_1} - 1)(e^{-\theta r_2} - 1)}{e^{-\theta} - 1}\right) \quad (13)$$
$$\theta \in (-\infty, \infty)\backslash\{0\}$$

式 (13) 中，C_θ^{Fr} 表示 Frank copula 函數，θ 為 Copula 函數之相依性參數。

隨機變數相依性分析

1. Kendall's tau (τ)

由於傳統探討變數相關性係以 Pearson 相關係數為指標，但 Pearson 相關係數僅表示兩變數間線性關係的程度高低，而無法呈現非線性關係或者共同變化的趨勢。掌握水稻產量及價格高低變化趨勢，攸關是否能準確評估收入風險，因此本研究使用 Kendall's tau 作為在給定 Copula 函數情況下，評估隨機變數變化趨勢一致性的指標。Kendall's tau 的定義如次所示 (Nelsen 2006)：

$$\begin{aligned}\tau &= \tau_{X,Y} \\ &= P\left[(X_1 - X_2)(Y_1 - Y_2) > 0\right] - \\ &\quad P\left[(X_1 - X_2)(Y_1 - Y_2) < 0\right]\end{aligned} \quad (14)$$

式 (14) 中，(X_1, Y_1) 與 (X_2, Y_2) 為獨立且相同分布之隨機向量，τ 表示為兩變數變化趨勢相同的機率減去變化趨勢不同的機率。

Kendall's tau 由 Copula 函數的導出式如次 (Schweizer & Wolff 1981)：

$$\tau_{X,Y} = \tau_C = 4\int_0^1\int_0^1 C(u, v)dC(u, v) - 1 \quad (15)$$

式 (15) 中，C 為連續隨機變數 X 與 Y 的 Copula 函數，令 $u = F(x)$ 與 $v = G(y)$，根據機率積分變換，u、v 係服從區間 [0, 1] 上的均勻分布。

2. 尾部相依係數 λ_U 及 λ_L

若隨機變數呈厚尾 (fat-tailed) 分布，表示出現極端值的機率將高於常態分布，因此為減少因發生系統性極端風險所造成的損失，必須評估隨機變數間尾部相關程度。尾部相關可分為上尾相依 (upper tail dependence) 與下尾相依 (lower tail dependence) 兩種情形，以水稻為例，若極端低產與低價的情況同步發生，對於收入將造成極大損失，因此就避險而言，下尾相依的影響更為重要。評估尾部相依分別以上尾相依係數 λ_U 與下尾相依係數 λ_L 表示如次 (Nelsen 2006)：

$$\begin{aligned}\lambda_U &= \lim_{i \to 1^-} P\left(Y > G^{-1}(i) \,\middle|\, X > F^{-1}(i)\right) \\ &= 2 - \lim_{i \to 1^-}\frac{1 - C(i, i)}{1 - i}\end{aligned} \quad (16)$$

$$\begin{aligned}\lambda_L &= \lim_{i \to 0^+} P\left(Y \le G^{-1}(i) \,\middle|\, X \le F^{-1}(i)\right) \\ &= \lim_{i \to 0^+}\frac{C(i, i)}{i}\end{aligned} \quad (17)$$

式 (16) 與式 (17) 中，F、G 表示隨機變數 X、Y 的機率分布函數，C 表示耦合 X、Y 的 Copula 函數。

分析步驟

1. 配適產量與價格之邊際機率分布

本研究共選取 Normal、Student's t、Weibull、Gamma、Beta、Log-normal、Logistic 及 Gumbel 8 種機率分布分別對於產量及價格進行配適，另以 Anderson-Darling test 進行適合度檢定 (goodness of fit) 評估配適結果，據此決定兩變數適當之邊際機率分布。

2. 配適耦合產量與價格之 Copula 函數

如前所述，本研究共選取 Gauss、*t*、Clayton、Gumbel 及 Frank 5 種 Copula 函數對於產量與價格進行耦合配適，另以赤池訊息量準則 (Akaike information criterion; AIC) 評估配適結果，據此決定適當之 Copula 函數耦合兩變數。

3. 構建產量與價格之聯合機率分布

首先根據 Step 2 已選定之 Copula 函數，應用機率積分變換原理，以蒙地卡羅方法模擬產生一組樣本數等於 10,000 之二維均勻分布隨機樣本 (U_{r_1}, U_{r_2}) 以建構 Copula 函數模板。接著再根據 Step 1 已選定兩變數之邊際機率分布 $(F_1(r_1), F_2(r_2))$，以 U_{r_1} 與 U_{r_2} 分別對於各邊際分布之累積分布函數 (cumulative distribution function; CDF) 計算其反函數值，求得一組 Copula 函數隨機樣本，如此則完成在 Copula 函數對於產量與價格邊際機率分布之耦合。

4. 水稻產量與價格相依性分析實證

本研究以歷年嘉義縣主要稻作生產鄉鎮市之第 1、2 期作產量與價格資料為基礎，透過對於兩變數之邊際分布機率配適與 Copula 函數耦合，產生 10,000 筆產量與價格模擬資料，兩者相乘後獲得收入分布模擬資料，並進一步估算其收入之期望損失 (expected loss; EL) 作為收入風險之指標，並以此進行不同期作與地區之比較分析。期望損失之估算方式如下 (Yang *et al*. 2021)：

$$EL = p(y < \alpha\hat{Y})\left(\alpha\hat{Y} - E(y|y < \alpha\hat{Y})\right) \quad (18)$$

式 (18) 中，EL 為期望損失，$\hat{Y}$ 為期望收入，α 為可容忍收入損失程度，第 1 期作 α 設為 95%，第 2 期作 α 設為 90% (https://www.afna.gov.tw/view.php?theme=web_structure&id=4088)。

統計分析

本研究統計分析工作係以程式語言 “R” (4.3.1 版) 撰寫後編譯執行，使用 HP 濾波法將稻穀產量與價格去趨勢化，係調用套件 “mFilter” (Balcilar 2019) 之函數 “hpfilter”。對於產量與價格之機率分布進行配適，係調用套件 “fitdistrplus” (Delignette-Muller *et al*. 2023) 之函數 “fitdist”。對於耦合產量與價格邊際機率分布之 Copula 函數進行配適，係調用套件 “copula” (Hofert *et al*. 2023) 之函數 “fitCopula”。估算 Kendall’s tau 與尾部相關係數 λ_U、λ_L 係調用套件 “copula” 之函數 “tau” 與 “lambda”。另調用套件 “stats” (R Development Core Team 2023) 之函數 “AIC” 對於配適結果進行評估。建構產量與價格二維分布之 Copula 函數模板，係使用 “naomalCopula”、“tCopula”、“claytonCopula”、“gumbelCopula” 及 “frankCopula” 5 個函數。選擇適當之 Copula 函數模板模擬產生樣本數為 10,000 之二維均勻分布隨機樣本，係使用函數 “rCopula” 完成。

結果

產量與價格邊際機率分布配適

嘉義縣大林鎮等 13 個鄉鎮市之第 1、2 期作水稻產量邊際機率分布配適 Anderson-Darling 測驗結果如表 1 所示，根據測驗值最小代表配適最佳 (goodness of fit) 原則，第 1 期作產量配適為 Logistic 分布者有大林、六腳、水上、朴子、鹿草、新港、溪口及義竹 8 個鄉鎮市為最多；配適為 Student’s *t* 分布者有布袋、民雄及東石 3 個鄉鎮居次；配適為 Weibull 與 Log-normal 分布者分別有中埔與太保。第 2 期作 13 個鄉鎮市中僅有新港配適為 Logistic 分布，其他 12 個鄉鎮市均配適為 Weibull 分布。嘉義縣第 1、2 期作水稻價格邊際機率分布配適測驗結果如表 2 所示，第 1 期作價格配適為 Gumbel 分布，第 2 期作配適為 Weibull 分布。另依據產量與價格之配適邊際機率分布參數，模擬產生 10,000 筆樣本資料，其樣本分布之偏度 (skewness) 與峰度 (kurtosis) 表現如表 3 與表 4 所示。在分布偏度方面，13 個鄉鎮市第 1 期作產量樣本分布僅中埔鄉偏度為負值呈現左偏態，其他鄉鎮市偏度均為正值呈現右偏態，價格樣本分布亦呈現右偏態。第 2 期作產量樣本分布僅新港為右偏態，其他均為左偏態，價格樣本分布亦呈現左偏態。在分布

表 1. 嘉義縣鄉鎮市水稻產量配適邊際機率分布 Anderson-Darling 測驗結果。

Table 1. The Anderson-Darling test results for fitting marginal probability distributions of rice yield in townships and cities in Chiayi County.

Area	Probability distribution							
	Normal	Student's *t*	Weibull	Gamma	Beta	Log-normal	Logistic	Gumbel
1st crop season								
Dalin	0.2469[z] (0.9719)[x]	0.2512 (0.9696)	0.4725 (0.7725)	0.2414 (0.9747)	3.9175 (0.0099)	0.2408 (0.9750)	**0.2091**[y] **(0.9879)**	0.4460 (0.7999)
Jhongpu	0.4193 (0.8272)	0.3893 (0.8574)	**0.2431** **(0.9739)**	0.4593 (0.7862)	2.0489 (0.0871)	0.4810 (0.7637)	0.3876 (0.8591)	0.6901 (0.5646)
Lioujiao	0.2117 (0.9871)	0.2103 (0.9875)	0.4455 (0.8004)	0.1898 (0.9932)	3.2937 (0.0199)	0.1829 (0.9946)	**0.1818** **(0.9949)**	0.2505 (0.9700)
Taibao	0.3697 (0.8765)	0.3601 (0.8856)	0.6308 (0.6167)	0.3325 (0.9107)	3.0544 (0.0263)	**0.3178** **(0.9232)**	0.3212 (0.9204)	0.3380 (0.9058)
Shueishang	0.2425 (0.9742)	0.2343 (0.9781)	0.2005 (0.9905)	0.2736 (0.9561)	2.8078 (0.0350)	0.2916 (0.9437)	**0.1836** **(0.9945)**	0.6487 (0.6006)
Budai	0.2920 (0.9434)	**0.2534** **(0.9684)**	0.3015 (0.9363)	0.2941 (0.9418)	1.3760 (0.2090)	0.2959 (0.9405)	0.3036 (0.9346)	0.3374 (0.9064)
Minsyong	0.2259 (0.9817)	**0.2136** **(0.9864)**	0.3229 (0.9189)	0.2306 (0.9798)	2.7891 (0.0357)	0.2350 (0.9778)	0.2261 (0.9817)	0.3752 (0.8712)
Puzi	0.2866 (0.9473)	0.2827 (0.9500)	0.3772 (0.8693)	0.3047 (0.9338)	3.0905 (0.0252)	0.3167 (0.9241)	**0.2229** **(0.9830)**	0.6248 (0.6223)
Dongshih	0.1735 (0.9963)	**0.1615** **(0.9978)**	0.1922 (0.9927)	0.1867 (0.9939)	2.4627 (0.0526)	0.1943 (0.9921)	0.1691 (0.9969)	0.4178 (0.8287)
Lucao	0.5120 (0.7320)	0.5121 (0.7318)	0.6778 (0.5752)	0.5283 (0.7154)	4.5088 (0.0051)	0.5393 (0.7045)	**0.3765** **(0.8699)**	1.0171 (0.3471)
Singang	0.7378 (0.5257)	0.7367 (0.5266)	1.2653 (0.2435)	0.6855 (0.5686)	3.6272 (0.0137)	0.6621 (0.5887)	**0.4850** **(0.7597)**	0.6401 (0.6083)
Sikou	0.2674 (0.9601)	0.2469 (0.9719)	0.2917 (0.9436)	0.2847 (0.9486)	1.9345 (0.1006)	0.2954 (0.9409)	**0.2384** **(0.9762)**	0.5207 (0.7232)
Yijhu	0.3101 (0.9295)	0.3009 (0.9367)	0.4523 (0.7933)	0.3086 (0.9306)	2.9347 (0.0302)	0.3111 (0.9287)	**0.2802** **(0.9518)**	0.4964 (0.7479)
2nd crop season								
Dalin	0.9254[z] (0.3972)[x]	0.9244 (0.3978)	**0.4374**[y] **(0.8087)**	1.2300 (0.2559)	3.1781 (0.0228)	1.3942 (0.2038)	0.4692 (0.7759)	1.9329 (0.1008)
Jhongpu	0.6837 (0.5701)	0.6714 (0.5807)	**0.3823** **(0.8643)**	0.8911 (0.4180)	2.3023 (0.0638)	1.0090 (0.3512)	0.4225 (0.8239)	1.5099 (0.1743)
Lioujiao	0.2161 (0.9855)	0.2104 (0.9875)	**0.1332** **(0.9996)**	0.3109 (0.9288)	2.8749 (0.0323)	0.3703 (0.8759)	0.1742 (0.9961)	0.6069 (0.6388)
Taibao	0.5421 (0.7016)	0.5496 (0.6941)	**0.3026** **(0.9353)**	0.7333 (0.5293)	3.6604 (0.0132)	0.8432 (0.4489)	0.3175 (0.9235)	1.3446 (0.2182)
Shueishang	0.5933 (0.6516)	0.5863 (0.6584)	**0.3412** **(0.9030)**	0.7755 (0.4968)	2.7667 (0.0367)	0.8811 (0.4243)	0.3738 (0.8726)	1.4233 (0.1959)
Budai	0.6341 (0.6137)	0.6157 (0.6306)	**0.4219** **(0.8246)**	0.8009 (0.4783)	2.4854 (0.0512)	0.8950 (0.4156)	0.4499 (0.7958)	1.2367 (0.2535)
Minsyong	0.6382 (0.6100)	0.6484 (0.6009)	**0.2664** **(0.9607)**	0.8619 (0.4366)	3.5204 (0.0154)	0.9856 (0.3635)	0.3083 (0.9309)	1.5705 (0.1608)
Puzi	0.5296 (0.7142)	0.5248 (0.7190)	**0.2500** **(0.9703)**	0.7411 (0.5232)	3.1080 (0.0247)	0.8603 (0.4376)	0.3261 (0.9162)	1.2253 (0.2575)

表 1. 嘉義縣鄉鎮市水稻產量配適邊際機率分布 Anderson-Darling 測驗結果。(續)

Table 1. The Anderson-Darling test results for fitting marginal probability distributions of rice yield in townships and cities in Chiayi County. (continued)

Area	Normal	Student's *t*	Weibull	Gamma	Beta	Log-normal	Logistic	Gumbel
	Probability distribution							
Dongshih	0.4701 (0.7750)	0.4565 (0.7891)	**0.3413** **(0.9029)**	0.6312 (0.6163)	2.4833 (0.0513)	0.7289 (0.5328)	0.3575 (0.8881)	0.9919 (0.3602)
Lucao	0.5259 (0.7179)	0.5213 (0.7226)	**0.2673** **(0.9602)**	0.7202 (0.5398)	2.9273 (0.0304)	0.8324 (0.4562)	0.3417 (0.9025)	1.2587 (0.2458)
Singang	0.8292 (0.4584)	0.8047 (0.4755)	0.6148 (0.6315)	1.0322 (0.3396)	2.0116 (0.0913)	1.1519 (0.2858)	**0.6146** **(0.6317)**	1.6442 (0.1460)
Sikou	0.6996 (0.5567)	0.7006 (0.5559)	**0.2901** **(0.9448)**	0.9189 (0.4011)	3.5888 (0.0143)	1.0387 (0.3364)	0.3690 (0.8772)	1.5631 (0.1624)
Yijhu	0.2497 (0.9704)	0.2412 (0.9748)	**0.1897** **(0.9932)**	0.3418 (0.9024)	2.5676 (0.0464)	0.4028 (0.8440)	0.2157 (0.9857)	0.6221 (0.6247)

[z] The Anderson-Darling test results.
[y] Bold text indicates the smallest Anderson-Darling test statistic.
[x] *P*-value of the Anderson-Darling test.

表 2. 嘉義縣水稻價格配適邊際機率分布 Anderson-Darling 測驗結果。

Table 2. The Anderson-Darling test results for fitting marginal probability distributions of rice prices in Chiayi County.

Crop season	Normal	Student's *t*	Weibull	Gamma	Beta	Log-normal	Logistic	Gumbel
	Probability distribution							
1st	0.4972[z] (0.7471)[x]	0.4773 (0.7675)	0.8249 (0.4614)	0.4848 (0.7599)	2.1702 (0.0750)	0.4787 (0.7661)	0.4387 (0.8074)	**0.3765**[y] **(0.8700)**
2nd	0.6495 (0.5999)	0.5895 (0.6553)	**0.5682** **(0.6759)**	0.6578 (0.5925)	1.0152 (0.3481)	0.6624 (0.5885)	0.6251 (0.6219)	0.7280 (0.5335)

[z] The Anderson-Darling test results.
[y] Bold text indicates the smallest Anderson-Darling test statistic.
[x] *P*-value of the Anderson-Darling test.

表 3. 嘉義縣鄉鎮市水稻產量配適邊際機率分布參數。

Table 3. The parameters for fitting marginal probability distributions of rice yield in townships in Chiayi County.

Area	Probability distribution	Parameter		Skewness	Kurtosis
1st crop season					
Dalin	Logistic	8,157.9055[z]	247.0248[y]	0.0275	4.0058
Jhongpu	Weibull	18.7712[x]	7,883.9380[w]	-0.8550	4.1064
Lioujiao	Logistic	6,753.2440	315.4119	0.0275	4.0058
Taibao	Log-normal	8.8624[v]	0.0787[u]	0.2413	3.0678
Shueishang	Logistic	8,437.9100	264.4222	0.0275	4.0058
Budai	Student's *t*	141.3286[t]	-	0.0060	3.0491
Minsyong	Student's *t*	112.8383	-	0.0028	3.0648
Puzi	Logistic	7,641.8170	254.1730	0.0275	4.0058
Dongshih	Student's *t*	114.4495	-	0.0028	3.0640
Lucao	Logistic	7,532.5040	198.9129	0.0275	4.0058
Singang	Logistic	7,140.5940	200.5429	0.0275	4.0058

表 3. 嘉義縣鄉鎮市水稻產量配適邊際機率分布參數。(續)

Table 3. The parameters for fitting marginal probability distributions of rice yield in townships in Chiayi County. (continued)

Area	Probability distribution	Parameter		Skewness	Kurtosis
Sikou	Logistic	8,045.6630	283.7603	0.0275	4.0058
Yijhu	Logistic	6,951.4290	264.2431	0.0275	4.0058
2nd crop season					
Dalin	Weibull	11.9966	8,121.2794	-0.7213	3.7084
Jhongpu	Weibull	12.0602	5,815.2191	-0.7232	3.7135
Lioujiao	Weibull	8.5215	5,455.5023	-0.5817	3.3661
Taibao	Weibull	10.8052	6,661.4445	-0.6825	3.6057
Shueishang	Weibull	12.3041	6,353.5144	-0.7302	3.7328
Budai	Weibull	10.3423	6,237.1840	-0.6652	3.5620
Minsyong	Weibull	12.2728	7,835.4154	-0.7294	3.7304
Puzi	Weibull	9.6989	6,665.1765	-0.6389	3.4974
Dongshih	Weibull	8.4619	5,588.5665	-0.5784	3.3590
Lucao	Weibull	10.5373	6,408.2935	-0.6727	3.5807
Singang	Logistic	6,872.9000	427.7575	0.0275	4.0058
Sikou	Weibull	12.2765	7,718.4210	-0.7295	3.7307
Yijhu	Weibull	8.2055	6,932.6652	-0.5639	3.3278

z Parameter “location” of the Logistic distribution.
y Parameter “scale” of the Logistic distribution.
x Parameter “shape” of the Weibull distribution.
w Parameter “scale” of the Weibull distribution.
v Parameter “μ” of the Log-normal distribution.
u Parameter “σ” of the Log-normal distribution.
t Parameter “degrees of freedom” of the Student’s *t* distribution.

表 4. 嘉義縣水稻價格配適邊際機率分布參數。

Table 4. The parameters for fitting marginal probability distributions of rice prices in townships in Chiayi County.

Crop season	Probability distribution	Parameter		Skewness	Kurtosis
1st	Gumbel	21.6069z	0.3938y	1.1437	5.3064
2nd	Weibull	31.6321	23.2420	-0.9265	4.5785

z Parameter “location” of the Gumbel distribution.
y Parameter “scale” of the Gumbel distribution.

峰度方面，第 1、2 期作產量與價格樣本分布峰度值均大於 3，顯示具有厚尾分布現象。

Copula 函數配適

配適耦合水稻產量與價格二元變數 Copula 函數之 AIC 檢定結果如表 5 所示，根據 AIC 值最小配適最佳原則，第 1 期作配適為 Clayton copula 者有大林、六腳、太保、水上、布袋、朴子、東石、鹿草及新港 9 個鄉鎮市為最多；配適為 Gaussian copula 者有中埔、民雄及溪口 3 個鄉居次；配適為 Gumbel copula 者僅有義竹鄉。顯示第 1 期作 Copula 函數配適以 Clayton 與 Gumbel 等阿基米德族 Copula 函數居多，而 Gaussian 之橢圓族 Copula 函數較少。第 2 期作配適為 Gaussian copula 者有大林、太保、水上、朴子及溪口 5 個鄉鎮市為最多；配適為 *t* copula 者有中埔、民雄及新港 3 個鄉；配適為 Frank copula 者有東石、鹿草

表 5. 嘉義縣鄉鎮市配適 Copula 函數之 AIC 檢定結果。

Table 5. The Akaike information criterion (AIC) test results for fitting copula functions in townships in Chiayi County.

Area	Copula function				
	Gaussian	*t*	Clayton	Gumbel	Frank
1st crop season					
Dalin	1.4631	3.4631	**1.4320**[z]	2.0000	1.5748
Jhongpu	**-3.3300**	-1.3297	-0.5148	2.0000	-1.9770
Lioujiao	0.3570	2.3574	**0.2966**	2.0000	0.9188
Taibao	0.3478	2.3478	**-0.0999**	2.0000	0.8065
Shueishang	-0.3432	1.4983	**-1.1237**	2.0000	0.2459
Budai	0.1651	2.1659	**0.0500**	2.0000	0.9364
Minsyong	**1.2019**	3.2019	1.2333	2.0000	1.6110
Puzi	1.9926	3.9930	**1.9560**	2.0000	2.0000
Dongshih	**0.7338**	2.7341	0.2012	2.0000	1.0144
Lucao	1.7621	3.7621	**1.5896**	2.0000	1.7453
Singang	1.9988	3.9988	**1.9868**	2.0000	1.9995
Sikou	**1.9809**	3.3846	2.0481	1.9857	1.9885
Yijhu	1.7951	3.5947	2.0000	**0.7880**	1.9506
2nd crop season					
Dalin	**-1.7381**	0.2619	-1.6162	2.0000	-1.1734
Jhongpu	1.9632	**0.6534**	1.1871	1.9448	1.8536
Lioujiao	1.9868	2.2684	2.0641	**1.8926**	1.9944
Taibao	**-0.6816**	1.2800	0.8286	2.0000	-0.4410
Shueishang	**1.0474**	1.7469	2.1549	2.0000	1.0922
Budai	1.8287	2.5344	**1.7745**	1.9927	1.9331
Minsyong	1.8917	**0.9707**	1.9557	1.9799	1.9472
Puzi	**0.1702**	1.3544	1.4128	2.0000	0.2535
Dongshih	-1.5100	-0.6600	0.6178	2.0000	**-1.6225**
Lucao	-0.3492	0.6427	0.9393	2.0000	**-0.4984**
Singang	0.7684	**0.5172**	1.6866	2.0000	0.6753
Sikou	**1.3421**	3.1777	1.3465	2.0000	1.5891
Yijhu	-2.3661	-1.0348	-0.1833	2.0000	**-2.4959**

[z] Bold text indicates the smallest Akaike information criterion test statistic.

及義竹 3 個鄉；配適為 Gumbel copula 者有六腳鄉；配適為 Clayton copula 者有布袋鎮。顯示第 2 期作 Copula 函數配適情況與第 1 期作不同，以 Gaussian 與 *t* 等橢圓族 Copula 函數較多，而 Frank、Clayton 及 Gumbel 等阿基米德族 Copula 函數較少。各鄉鎮市配適 Copula 函數之參數、產量及價格之 Kendall's tau 相關係數 τ 與上下尾部相關係數 λ_U、λ_L 如表 6 所示，Kendall's tau 相關係數在第 1 期作除義竹鄉之 0.1817 為正相關外，其他 12 個鄉鎮市均呈負相關，其中負相關係數最高者為中埔鄉之 -0.4284，較低者為朴子市之 -0.0167、溪口鄉之 -0.0333、新港鄉之 -0.0449 及鹿草鄉之 -0.0833，呈輕微負相關。第 2 期作除中埔

表 6. 嘉義縣鄉鎮市配適 Copula 函數參數與 Kendall’s tau 相關係數。

Table 6. The parameters for fitting copula functions and Kendall rank correlation coefficient in townships in Chiayi County.

Area	Copula function	Parameter	τ^z	$\lambda_U{}^y$	$\lambda_L{}^x$
1st crop season					
Dalin	Clayton	-0.2090	-0.1167	0	0
Jhongpu	Gaussian	-0.6233	-0.4284	0	0
Lioujiao	Clayton	-0.2609	-0.1500	0	0
Taibao	Clayton	-0.3562	-0.2167	0	0
Shueishang	Clayton	-0.3784	-0.2333	0	0
Budai	Clayton	-0.3099	-0.1833	0	0
Minsyong	Gaussian	-0.3063	-0.1982	0	0
Puzi	Clayton	-0.0328	-0.0167	0	0
Dongshih	Clayton	-0.2857	-0.1667	0	0
Lucao	Clayton	-0.1538	-0.0833	0	0
Singang	Clayton	-0.0860	-0.0449	0	0
Sikou	Gaussian	-0.0523	-0.0333	0	0
Yijhu	Gumbel	1.2220	0.1817	0.2366	0
2nd crop season					
Dalin	Gaussian	-0.5542	-0.3739	0	0
Jhongpu	*t*	0.1533	0.0980	0.1135	0.1135
Lioujiao	Gumbel	1.0803	0.0743	0.1004	0
Taibao	Gaussian	-0.4931	-0.3282	0	0
Shueishang	Gaussian	-0.3299	-0.2140	0	0
Budai	Clayton	-0.0952	-0.0500	0	0
Minsyong	*t*	-0.0527	-0.0336	0.0650	0.0650
Puzi	Gaussian	-0.4278	-0.2814	0	0
Dongshih	Frank	-3.5360	-0.3522	0	0
Lucao	Frank	-2.8867	-0.2973	0	0
Singang	*t*	-0.3631	-0.2366	0.0222	0.0222
Sikou	Gaussian	-0.2821	-0.1821	0	0
Yijhu	Frank	-3.9509	-0.3845	0	0

z Kendall’s τ coefficient.
y Upper tail dependence parameter.
x Lower tail dependence parameter.

鄉之 0.0980 與六腳鄉之 0.0743 呈輕微正相關外，其他 11 個鄉鎮市亦均呈負相關，其中負相關係數最高者為義竹鄉之-0.3845，較低者為民雄鄉之-0.0366 與布袋鎮之-0.0500，呈輕微負相關。上尾相關係數 λ_U 在第 1 期作僅義竹鄉為 0.2366，其餘鄉鎮市皆為 0；第 2 期作則有中埔鄉為 0.1135、六腳鄉為 0.1004、民雄鄉為 0.0650 及新港鄉為 0.0222。下尾相關係數 λ_L 在第 1 期作各鄉鎮市皆為 0；第 2 期作則有中埔鄉為 0.1135、民雄鄉為 0.0650 及新港鄉為 0.0222。

模擬結果

在水稻產量與價格之邊際機率分布，以及耦合二元變數之 Copula 函數均已確定之情況下，模擬各鄉鎮市水稻樣本產量與價格之

Kendall's tau 相關係數如表 7 所示，比較實際資料與模擬資料之相關係數，第 1 期作除義竹鄉之實際資料相關係數為 0，模擬資料相關係數為 0.1871 之外，其他鄉鎮市之實際資料與模擬資料之相關趨勢均為負相關。實際資料與模擬資料相關係數差距取絕對值後較大者依次為義竹鄉、中埔鄉、民雄鄉、新港鄉及溪口鄉。

表 7. 嘉義縣鄉鎮市水稻實際產量及價格資料與模擬資料之 Kendall's tau 相關係數比較。

Table 7. Comparison of Kendall's tau between actual and simulated data on rice yield and price in townships and cities of Chiayi County.

Area	τ_a^z	τ_s^y
1st crop season		
Dalin	-0.1167	-0.1170
Jhongpu	-0.3333	-0.4266
Lioujiao	-0.1500	-0.1501
Taibao	-0.2167	-0.2162
Shueishang	-0.2333	-0.2328
Budai	-0.1833	-0.1832
Minsyong	-0.1167	-0.1963
Puzi	-0.0167	-0.0176
Dongshih	-0.1667	-0.1667
Lucao	-0.0833	-0.0840
Singang	-0.0167	-0.0458
Sikou	-0.0167	-0.0315
Yijhu	0.0000	0.1871
2nd crop season		
Dalin	-0.2833	-0.3721
Jhongpu	0.0833	0.0963
Lioujiao	0.0333	0.0833
Taibao	-0.2667	-0.3263
Shueishang	-0.1667	-0.2120
Budai	-0.0500	-0.0508
Minsyong	-0.0500	-0.0345
Puzi	-0.2167	-0.2794
Dongshih	-0.3000	-0.3543
Lucao	-0.2500	-0.2995
Singang	-0.2500	-0.2355
Sikou	-0.1333	-0.1801
Yijhu	-0.3500	-0.3865

z Kendall's tau of the actual data.
y Kendall's tau of the simulated data.

第 2 期作各鄉鎮市實際資料與模擬資料之相關趨勢均表現一致，除中埔鄉與六腳鄉為正相關外，其他各鄉鎮市均為負相關。實際資料與模擬資料相關係數差距取絕對值後較大者依次為大林鎮、朴子市、太保市、東石鄉、六腳鄉、鹿草鄉、溪口鄉、水上鄉、義竹鄉、民雄鄉、新港鄉及中埔鄉。

模擬收入分布之敘述統計量及期望損失估算結果如表 8 所示，第 1 期作分布偏度除民雄鄉及中埔鄉為負值呈左偏態，其他各鄉鎮市均為正值呈右偏態，其中右偏最大值為太保市之 0.4535，最小為溪口鄉之 0.0441。各鄉鎮分布峰度值一致表現大於 3 以上，呈厚尾分布型態，其中峰度值最大為中埔鄉之 4.6246，最小為民雄鄉之 3.0874。模擬收入樣本分布平均值最高為水上鄉之 184,006 NT$ ha^{-1}，最低為東石鄉之 143,191 NT$ ha^{-1}。模擬收入樣本分布全距最大為義竹鄉之 138,179 NT$ ha^{-1}，最小為東石鄉之 63,464 NT$ ha^{-1}。模擬收入期望損失最高前三名依次為民雄鄉之 2,219.6 NT$ ha^{-1}、六腳鄉之 1,849.7 NT$ ha^{-1} 及義竹鄉之 1,723.2 NT$ ha^{-1}；期望損失最低前三名依次為鹿草鄉之 600.6 NT$ ha^{-1}、新港鄉之 725.2 NT$ ha^{-1} 及東石鄉之 778.8 NT$ ha^{-1}。

第 2 期作模擬收入樣本分布型態與第 1 期作有所不同，各鄉鎮市分布偏度均為負值呈左偏態，其中左偏最大值為新港鄉之-0.0854，最小值為大林鎮之-0.7979。各鄉鎮分布峰度一致表現大於 3 以上，呈厚尾分布型態，其中峰度值最大為新港鄉之 4.3788，最小為布袋鎮之 3.2746。模擬收入樣本分布平均值除民雄鄉及新港鄉外，其他鄉鎮市均較第 1 期作低，其中平均最高為大林鎮之 177,277 NT$ ha^{-1}，最低為六腳鄉之 117,353 NT$ ha^{-1}。模擬收入樣本分布全距最大為新港鄉之 167,460 NT$ ha^{-1}，最小為水上鄉之 108,132 NT$ ha^{-1}。模擬收入期望損失除民雄鄉之 2,091.9 NT$ ha^{-1} 較第 1 期作減少外，其他鄉鎮市均較第 1 期作增加，期望損失最高前三名依次為六腳鄉之 2,919.4 NT$ ha^{-1}、義竹鄉之 2,844.4 NT$ ha^{-1} 及東石鄉之 2,203.9 NT$ ha^{-1}；最低前三名依次為水上鄉之 1,349.3 NT$ ha^{-1}、大林鎮之 1,497.3 NT$ ha^{-1} 及新港鄉之 1,630.3 NT$ ha^{-1}。

表 8. 嘉義縣鄉鎮市模擬水稻收入分布與期望損失。

Table 8. The simulation of rice income and expected loss in townships in Chiayi County.

Area	Income (NT$ ha^{-1})							EL^z (NT$ ha^{-1})
	Mean	Median	Maxium	Minimum	Range	Skewness	Kurtosis	
1st crop season								
Dalin	177,935	177,426	229,034	134,176	94,858	0.2871	3.9599	867.5
Jhongpu	167,066	168,335	189,375	109,769	79,606	-0.9617	4.6246	1,237.2
Lioujiao	147,212	146,684	210,324	91,396	11,8928	0.1909	4.0453	1,849.7
Taibao	154,384	153,322	207,549	120,780	86,769	0.4535	3.2735	1,545.6
Shueishang	184,006	183,303	237,569	140,789	96,780	0.3980	4.1281	780.8
Budai	150,790	150,210	194,520	115,494	79,026	0.2431	3.1307	1,315.7
Minsyong	159,267	159,174	209,585	104,247	105,338	-0.0167	3.0874	2,219.6
Puzi	166,691	166,356	219,628	118,291	101,337	0.1583	3.9053	1,195.3
Dongshih	143,191	142,748	179,100	115,636	63,464	0.2947	3.1848	778.8
Lucao	164,320	163,899	206,203	128,160	78,043	0.2947	3.9098	600.6
Singang	155,771	155,422	198,011	118,076	79,935	0.2273	3.8896	725.2
Sikou	175,553	175,542	234,391	109,775	124,616	0.0441	4.0528	1,503.7
Yijhu	151,525	151,140	224,592	86,413	138,179	0.3171	4.5452	1,723.2
2nd crop season								
Dalin	177,277	179,003	217,718	89,722	127,996	-0.7979	4.2233	1,497.3
Jhongpu	127,262	128,619	168,730	55,524	113,206	-0.5872	3.8166	1,821.4
Lioujiao	117,353	118,637	170,944	32,778	138,166	-0.4244	3.3069	2,919.4
Taibao	144,812	146,369	182,718	65,953	116,765	-0.7333	3.9946	1,656.8
Shueishang	138,925	140,235	174,165	66,033	108,132	-0.6596	3.8310	1,349.3
Budai	135,451	136,533	182,398	63,234	119,164	-0.4177	3.2746	2,064.2
Minsyong	171,451	173,323	225,389	78,713	146,676	-0.6112	3.9478	2,091.9
Puzi	144,236	145,911	187,723	58,158	129,565	-0.6667	3.7838	2,184.5
Dongshih	120,032	121,437	166,398	47,779	118,619	-0.5824	3.7085	2,203.9
Lucao	139,129	140,504	183,560	66,110	117,450	-0.6119	3.8441	1,680.3
Singang	156,623	156,746	239,387	71,927	167,460	-0.0854	4.3788	1,630.3
Sikou	168,781	170,386	212,643	78,940	133,703	-0.6403	3.7717	1,714.1
Yijhu	148,635	150,436	207,142	57,479	149,663	-0.5937	3.7276	2,844.4

[z] Expected loss.

討論

本研究透過 Copula 函數分析水稻產量及與價格之相依性結果，分別就 Kendall’s tau 相關係數、尾部相關性及不同期作與地區之收入風險分布情形討論如次。首先，就產量及價格之 Kendall’s tau 相關係數表現而言，比較各鄉鎮市水稻產量與價格實際資料及模擬資料之相關係數，顯示兩者相關趨勢均表現一致。在相關係數差距大小方面，將相關係數取絕對值後，大致以模擬資料略高於實際資料，惟差距甚微，顯示透過 Copula 函數耦合後之模擬資料仍維持實際資料之相依結構。第 1 期作除義竹鄉、第 2 期作中埔鄉及六腳鄉表現為正相關外，其他大多數鄉鎮市在第 1、2 期作均呈現負相關。在市場機制下，產量與價格負相關顯示兩者風險藉此得以對沖，可平抑部分收入風險。惟本研究中各鄉鎮市之 Kendall’s tau 相

關係數高低與期望損失大小之間並無明顯對應關係，顯示市場機制仍不足以充分解釋價量變化，致使相關係數偏低，相關性表現微弱，因此市場價量風險對沖效果，無法充分反應在平抑收入風險的表現上。

其次，在風險管理上，尾部相關用以評估 2 個變數同時出現極端值機率的大小，特別應減少下尾相關的發生機率，避免造成系統性的損失巨災。以水稻而言，若於成熟期遭遇颱風或豪雨造成稻株倒伏或穗上發芽，或者抽穗期遭遇高溫造成產量下降與稻米外觀品質劣化，則可能同時發生低產與低價的情況。本研究藉由 Copula 函數分析水稻產量與價格之間的尾部相關性，第 1 期作除義竹鄉有上尾相關外，其他各鄉鎮市皆無尾部相關；第 2 期作則有六腳鄉為上尾相關，而中埔、民雄及新港鄉則為對稱尾部相關，其他 11 個鄉鎮市皆無尾部相關，顯示各鄉鎮市在兩期作同時發生低產與低價極端事件，造成收入嚴重損失的機率較低。但值得注意的是，第 1 期作各鄉鎮市收入與價格耦合配適之 Copula 函數，大多數為對於下尾相關較為敏感之 Clayton copula。由於近年第 1 期稻作生產經常由於孕穗開花期之高溫乾旱，以及成熟期遭遇連續降雨或短延時強降雨等極端天氣影響，造成水稻減產 (Peng *et al*. 2004)、稻米白堊質粒及胴割等品質劣化現象 (Resurreccion *et al*. 1977; Chao 1992)。以中央氣象署設於嘉義縣溪口鄉之農試溪口農場農業氣象站 (23°35'N, 120°24'E) 資料 (https://codis.cwa.gov.tw/StationData) 為例，2018–2023 年第 1 期作水稻孕穗期 (約 4 月下旬) 之前平均累計降水量 (累計區間自前一年 9 月起至當年 4 月止) 為 217.7 mm，而 2021 年與 2023 年則分別僅有 154 mm 與 133 mm；5 月抽穗開花期平均氣溫為 26.4℃，而 2021 年與 2023 年則為 27.9℃與 26.3℃，均超過水稻高溫逆境定義值 26.0℃ (Lur *et al*. 2006)，因此造成低產與低價同時發生下尾相關的潛在風險仍不可忽視。

最後，模擬各鄉鎮市兩期作之收入期望損失估算研究結果顯示，比較不同期作別之期望損失，則除了民雄鄉外，其他鄉鎮市皆以第 2 期作之期望損失高於第 1 期作，此係第 2 期作水稻生育初期天氣不穩定，以及生育後期日射量較低影響產量，造成稻作生產風險較高 (Tsai *et al*. 1984)。比較各鄉鎮市期望損失高低，發現六腳鄉與義竹鄉在兩期作均列名前 3 名內，收入風險較高；而新港鄉在兩期作則均列名末 3 名內，收入風險較低。同時也發現期望損失與期望收入全距大小亦大致對應，表示期望收入高低差距過大之地區，收入風險亦同時增加。至於六腳鄉與義竹鄉之收入風險較高，推測可能因沿海地區稻作栽培環境較為嚴苛，收入波動幅度較大所致。Singh *et al*. (2017) 透過對於印度雨養水稻 (rainfed rice) 栽培地區，因氣候變遷帶來高溫與旱澇等逆境造成稻作生產風險模擬評估之研究，建議在高風險稻作產區，可藉由推廣栽培調適技術、開發耐逆境新品種及對於生產高風險地區的政策支持等措施，提高稻作生產韌性，降低農民收入風險。Loko *et al*. (2022) 探討非洲貝南共和國稻作生產的限制因子，同樣建議推廣現代栽培技術與採用新品種，可提高稻作產量。因此開發適應環境的水稻新品種與韌性栽培技術，在增加及穩定農民收益方面，深具積極投入的研究價值。

透過 Copula 函數耦合水稻產量與價格變數，除可簡化推算兩變數之聯合機率分布，同時深入分析兩變數之 Kendall's tau 相依性與尾部相關，以作為評估收入風險之參考。實際上，本研究採用之 5 種 Copula 函數中僅有 Clayton 與 Gumbel 函數可偵測下尾與上尾等尾部相關。為能更全面偵測各種尾部相關，可進一步研究使用混合 Copula 函數估算尾部相關問題。此外，目前價格資料尺度因受限於資料來源，僅有縣市層級資料而未及於鄉鎮層級，因此無法與產量資料完全對應，可能對於各地區水稻收入風險分布分析結果造成影響，未來應設法建立以鄉鎮市為單位的價格資料，俾使收入風險分布更接近實際情況。

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Study on the Dependency Analysis of Rice Yield and Price Using Copula Function

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Abstract

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The main objective of this study is to analyze the dependency between yield and price variables of rice cultivation in the main townships of Chiayi County through Copula functions, in order to estimate income risks and provide references for rice production adaptation measures. The analysis results indicate that, except for the first cropping season in Yizhu Township and the second cropping season in Zhongpu Township and Liujiao Township, the Kendall's tau correlation coefficients between rice yield and price in other townships during both cropping seasons show a negative correlation. However, the correlation coefficients are relatively low, indicating weak correlation. In terms of tail dependence, the first cropping season in Yizhu Township shows upper tail dependence, while the second cropping season in Zhongpu, Minxiong, and Xingang Townships exhibit symmetric tail dependence, and Liujiao Township shows upper tail dependence. The other townships display no significant tail dependence or lower tail dependence in both cropping seasons. Notably, for the first cropping season, most townships exhibit yield and price coupling fitted with the Clayton Copula function, which is sensitive to lower tail dependence. Due to the frequent impact of extreme weather during the late growth stage of the first cropping season in recent years, the potential risk of lower tail dependence, where both low yield and low price occur simultaneously, cannot be ignored. Lastly, simulated expected losses in rice income for each township were calculated to assess income risk. The findings reveal that, except for Minxiong Township, the expected losses during the second cropping season are higher than those in the first cropping season. This is possibly related to meteorological disasters during the early growth stage of the second cropping season and lower solar radiation in the later stages, which increases production risks. When comparing the expected losses across townships, Liujiao and Yizhu Townships show higher losses in both cropping seasons, likely due to the harsher rice cultivation environment in coastal areas, leading to greater income volatility. Future efforts may focus on developing adaptive cultivation technologies or breeding new stress-resistant varieties to enhance the resilience of rice production in coastal areas and reduce income risks.

Key words: Rice, Copula function, Dependence.

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研究報告

臺灣水稻稻麴病之發生與防治藥劑之篩選

賴柏羽[1] 吳昭蓉[1] 倪蕙芳[2,*]

摘要

賴柏羽、吳昭蓉、倪蕙芳。2025。臺灣水稻稻麴病之發生與防治藥劑之篩選。台灣農業研究 74(1):63–79。

水稻稻麴病 (*Ustilaginoidea virens*) 為臺灣水稻次要病害，偶於田間發生，近年來隨著友善耕種觀念普及，水稻有機栽培面積逐年增加，加上受到氣候變遷影響，稻麴病在世界各地均有越來越頻繁且嚴重發生之趨勢，除了影響水稻產量與品質外，其產生之毒素亦可能威脅稻米品質與安全。由於本病害在臺灣發生之相關報告極為有限，本研究於 2022–2023 年在嘉義縣民雄鄉有機水稻田進行 2 年 4 期作之水稻稻麴病害發生率調查，結果顯示稻麴病分別於 2022 年 1 期稻作與 2023 年 2 期稻作發生，其中又以 2022 年病害發生率較為嚴重，稻欉罹病率最高達 27.5–30.7%，罹病穗率為 9.6–10.6%，罹病穗之稻麴數以 1–2 粒占多數，且多分布於稻穗中下段。另外，自田間所分離之 9 株稻麴病菌菌株，以 potato sucrose agar (PSA) 培養基培養時，其產孢能力、菌落型態及生長速度彼此間具有差異性，其中 6 菌株在 2–4 wk 陸續產生厚膜孢子，但有 3 菌株則完全不產生孢子。進一步挑選 6 株菌落型態不同之菌株進行核酸定序，結果顯示，6 菌株之 internal transcribed spacer (ITS) 序列彼此相同，而與美國國家生物科技資訊中心 (National Center for Biotechnology Information; NCBI) 基因資料庫比對結果則與 *U. virens* 菌株 118-CLR-KA-40 的 ITS 序列 (Accession no. OL744511) 有高達 99.69% 之相似度，鑑定本菌為 *U. virens*。以交配型基因座 (mating type locus) 之專一性引子對增幅，發現本研究所收集的菌株為 MAT1-1 交配型，並未發現有 MAT1-2 交配型的存在。另挑選其中產孢較穩定之 RSB-5 與 RSB-9 分離株進行溫度試驗，結果顯示 25℃為最適合菌絲生長與厚膜孢子發芽之溫度條件。以目前推薦在水稻真菌性病害防治之藥劑對 RSB-5 與 RSB-9 進行室內菌絲生長與孢子發芽抑制試驗，結果顯示百克敏、鋅錳乃浦、克熱淨及免賴得對供試菌株之菌絲生長抑制率最佳，嘉賜黴素抑制率最低；百克敏、甲基多保淨、鋅錳乃浦、克熱淨及免賴得對厚膜孢子具有 100% 發芽抑制率，而嘉賜三賽唑與嘉賜黴素則抑制效果低。綜合以上結果，在水稻孕穗期以百克敏、鋅錳乃浦、克熱淨及免賴得等殺菌劑輪流施藥防治，應可減低稻麴病的危害。

關鍵詞：水稻、稻麴病、病害防治。

前言

水稻稻麴病 (rice false smut)，又稱為『稻 (穀) 母』(Sawada 1912)，是由 *Ustilaginoidea virens* (Cooke) P. Henn 所引起的真菌病害，本病原菌屬子囊菌門 (Ascomycota)，肉座菌目 (Hypocreales)，麥角菌科 (Clavicipitaceae)，稻麴病菌屬 (*Ustilaginoidea*) 之真菌 (Kepler *et al*. 2012)。此病原菌最早發現於 1878 年 (Cooke 1878) 並命名為 *Ustilago virens*，Brefeld (1895) 基於分生孢子特徵建立新屬 *Ustilaginoidea*，並將本菌定名為 *U. oryzae*，Takahashi (1896) 將學名合併為 *U. virens*。Sakurai (1934) 首次報導由稻麴上菌核發現本菌之有性態，Nakata (1934) 將本菌之有性態定名為 *Claviceps virens*。然而，系統發育分析顯示 *Ustilaginoidea* 不屬於 *Claviceps* (Bischoff *et al*. 2004; Tanaka & Tanaka 2008; Tanaka 2015)，一直至

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Tanaka *et al*. (2008) 建立新屬 *Villosiclava* 並將有性態學名合併為 *V. virens*，就此確立稻麴病菌之分類地位。

本病害最明顯之特徵為成熟穀粒呈病理性膨大而形成『稻麴』(smut ball)，病粒大小約為健全穀粒 3–4 倍大，成熟之稻麴表面薄膜破裂後，露出黃綠色或黃黑色的厚膜孢子 (Kim & Park 2007)，有時夾雜有性世代之菌核 (Sun *et al*. 2020)。然而稻麴病菌可能在水稻孕穗期或更早之前便已由穎穀間隙完成入侵 (Tanaka 2015)，主要感染穎花 (Ashizawa *et al*. 2012)，在感染初期於穎殼內生長形成淡黃色的菌絲塊，使內外穎膨脹，並隨穀粒成熟由縫合處露出灰綠色的菌塊，逐漸包裹整個穎殼，呈黃、黃綠及綠色等，最後形成稻麴之病徵。

稻麴病最初在印度的稻穀發現 (Cooke 1878)，隨後於日本、菲律賓及馬來西亞等亞洲國家皆有報導 (Patouillard 1887; Graff 1918)。本病多為零星發生，但在部分地區仍有偶發性大發生的案例，直至 1980 年代之後，隨著高產量水稻品種的種植與推廣，以及化學肥料用量不斷增加，加上近年氣候變遷等因素，導致稻麴病之發生已涵蓋亞洲、美洲、非洲、南美洲及歐洲等 61 個國家之水稻產區 (CABI 2021)，並持續有新發生紀錄的報告 (Atia 2004)，且有越來越嚴重的趨勢，日本、印度及中國等地便時常有稻麴病大發生之報告 (Matsumoto & Ibaraki 1981; Dodan & Singh 1996; Sasahara *et al*. 2008a)，發病面積明顯提升，以日本為例，近 10 年間，日本稻麴病發生面積已超過 9 萬公頃 (NARO 2023)；印度則在 2009–2010 年平均發病率曾高達 76.7%，單穗上最多達 30 個稻麴之紀錄 (Ladhalakshmi *et al*. 2012)。

臺灣最早於 1908 年正式記錄稻麴病的發生 (Kawakami & Suzuki 1908)，1907–1916 年間於臺北至屏東地區皆有分布 (Sawada 1919)，係當時 12 種重要稻作病害之一，其中又以嘉義地區發生較多 (Sawada 1912)。在此之後，此病害零星發生，嚴重程度不及其他水稻重要病害，分別於 1967 與 1990 年僅有 2 篇系統性的調查，闡明當時田間發病率可高達 19.3% (Chou 1967; Tsai *et al*. 1990)。然而，其危害不僅局限於罹病稻的損失，也包括不稔率增加與千粒重減少所造成之產量損失 (Iijima & Konuma 1983)。此外，死米率增加與稻麴上產生的大量孢子在碾米過程中污染了機械與正常稻米，也是使品質降低的重要原因之一 (Osada 1995)；更甚者，被感染的莖葉或稻穗等罹病組織中存在多種真菌毒素 (mycotoxin)，主要成分為 ustiloxin 與 ustilaginoidins (Yabuta & Sumiki 1933; Koiso *et al*.1992, 1994; Meng *et al*. 2015)，導致動植物細胞分裂受到抑制，具廣泛之生物毒性，嚴重威脅稻米食用安全與人畜健康 (Miyazaki 2006; Miyazaki *et al*. 2009; Sun *et al*. 2017)，也因此更需要瞭解田間有關此病害之發生情形與病原菌之特性。

目前在臺灣針對此病害相關研究相當少，且無任何推薦藥劑，而近年氣候變遷與友善耕作面積增加，稻麴病已在臺灣時有嚴重發生之現象，對未來之發生風險更無法預測，因此本研究除進行稻麴病發生調查外，並針對稻麴病菌之病原特性與可能防治藥劑進行篩選，期能研發有效防治策略與方法供農友參採。

材料與方法

試驗田區

供試水稻田位於嘉義縣民雄鄉共 2 區 (A: 23°34'02.8"N 120°27'16.2"E; B: 23°34'02.9"N 120°27'15.4"E)，距離相鄰約 100 m，皆為有機認證田區，為確實與周邊慣行水稻田作阻隔，供試田區在田埂周圍種植高度達 1 m 以上之灌木綠籬。水稻每年皆種植 2 期作，第一期作種植品種為「台農秈 22 號」(‘TNS 22’)，第二期作種植品種為「高雄 147」(‘KH 147’)，種植密度為行株距 30 × 24 cm。於 2019–2023 年間，每隔 2–3 d 記錄水稻自插秧至收割之各生育時期，並參考交通部中央氣象署自動氣象站民雄測站 (測站代碼：C0M760) 記錄水稻不同生育期間之日均溫、相對濕度及每日降雨量等氣象資料 (CODiS 氣候觀測資料查詢服務，https://codis.cwa.gov.tw)。

病害調查

供試田區於 2022 年 1 期稻作期間發現嚴重之稻麴病害，因此於 2022–2023 年間，連

續調查4期稻作之病害發生情形。在水稻進入糊熟期至黃熟期階段，參考 Tsai *et al*. (1990) 之田間調查方式，將田區由中央劃分為4小區，每小區調查2對角線，記錄每欉水稻穗數，並依據下列公式計算罹病欉率及罹病穗率：罹病欉率 (%) = (罹病稻欉數/調查稻欉總數) × 100%；罹病穗率 (%) = (罹病穗數/罹病欉之稻穗總數) × 100%。另記錄罹病穗上形成稻麴之數量，並將病穗平均分為上、中、下段，記錄稻麴在病穗之分布位置。

稻麴病菌的分離與培養

本研究於 2022 年自供試田區採集具有稻麴病病徵之稻穗，將稻穗病徵上已產孢之黃色厚膜孢子堆以適量無菌水製成孢子懸浮液，吸取 20 μL 孢子懸浮液，以劃線平板法將孢子懸浮液劃線於 2% Bacto agar (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) 培養基上，以 25℃培養 48 h 後，於解剖顯微鏡下切取已發芽的單孢至 potato sucrose agar (PSA) (300 mL 馬鈴薯抽出液 (馬鈴薯 300 g 與 H_2O 1,000 mL) 加入6 g sucrose (Merck & Co., Inc., Kenilworth, NJ, USA) 及 6 g agar (惠昇食品股份有限公司，臺灣彰化縣)) 培養基，於 25℃培養約 3 wk 後，切取菌絲塊，保存於無菌水小管並儲存於 10℃定溫箱備用。

稻麴病菌之型態觀察

將 9 株稻麴病菌分離株 (RSB-1 至 RSB-9) 活化於 PSA 培養基後，以直徑 0.5 cm 滅菌之打孔器切取菌絲邊緣，分別移植於 PSA 培養基，於 25℃下黑暗培養 4 wk，調查菌落平均直徑。因 RSB-5 與 RSB-9 產孢較穩定，取二者產生之厚膜孢子置於載玻片上，以干涉位像差顯微鏡 (differential interference contrast, DIC; Nikon 80i, Nikon Corporation, Tokyo, Japan) 觀察，再以顯微鏡照相機 (Gryphax Naos, Jenoptik, Jena, Germany) 拍照後，並以其所附之軟體測量 50 個厚膜孢子大小。

為更進一步觀察厚膜孢子之表面，本研究另以掃描式電子顯微鏡 (scanning electron microscope, SEM; TM-1000, Hitachi, Ltd., Tokyo, Japan) 拍攝稻趨菌厚膜孢子。將稻趨菌培養於 PSA 一個月，待其產孢後，切下邊長 1 cm 產孢區域之菌塊，直接置於電子顯微鏡之載臺上，以電子顯微鏡附屬之影像軟體拍攝圖片及顯示尺規。

稻麴病菌之分子鑑定及交配型基因座分析

將 RSB-1、RSB-2、RSB-3、RSB-5、RSB-6 及 RSB-9 分離株培養於 PSA 培養基，待長出足量菌絲後，刮取少量菌絲，置於 50 μL Magic Quick Seed Extract Solution (Bio-Genesis Technologies, Inc., Taipei, Taiwan) 中，以 65℃/6 min 與 95℃/2 min 處理後，作為後續 polymerase chain reaction (PCR) 反應中之 DNA 模板，並進行真菌內轉錄區間 ITS 部分序列、交配型基因座 (mating-type locus) MAT1-1 與 MAT1-2 之增幅。本試驗所使用之 PCR 溶液包含 5× PCR 緩衝液 (Fast-Run Taq Master Mis, Protech Technology Enterprise Co., Ltd., Taipei, Taiwan) 以及 0.5 μM primer，並加入無菌水使其總體積為 25 μL。所使用之 PCR 引子對與 PCR 條件如下：用於增幅 ITS 序列之 ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) 與 ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White *et al*. 1990)。反應條件為：94℃/4 min；接著以 94℃/30 s、54℃/30 s 及 72℃/45 s 進行 33 個循環；72℃/7 min。用於增幅 MAT1 locus 與 MAT2 locus 序列之引子對如下，MAT1-1 之引子對為 MAT1F2 (5′-GAAACTCCAACT-CAAACGAAGTCG-3′) 與 MAT1R2 (5′-GTA-AACTTTGGCTATCAACGCCC-3′)；而 MAT1-2 之引子對為 MAT2F2 (5′-GGAGCGACATA-ATACCGTCAAAGA-3′) 與 MAT2R2 (5′-GG-GGTGTTTTCTAAGAGGGCCT-3′) (Fu *et al*. 2014)。反應條件為：94℃/4 min；接著以 94℃/30 s、56℃/1 min 及 72℃/40 s 進行 35 個循環；72℃/7 min。PCR 產物以 1.5% Agarose gel 與 0.5× Tris/Borate/Ethylenediaminetetraacetic acid (TBE) 緩衝液進行核酸電泳分析，並於電泳前將 DNA 樣本與 EZ-vision DNA dye as loading buffer (Protech Technology Enterprise Co., Ltd., Taipei, Taiwan) 混合，以進行核酸染色及觀察。所得 PCR 產物則委託源資國際生物科技 (Tri-i Biotech, Inc., Taipei, Taiwan)

進行核酸定序，定序後之核酸序列利用美國國家生物科技資訊中心 (National Center for Biotechnology Information; NCBI) 基因資料庫進行序列查詢與比對。

溫度對稻麴病菌菌絲生長與孢子發芽之影響

稻麴病菌 RSB-5 與 RSB-9 分離株以 PSA 平板培養 4 wk 後，以直徑 0.5cm 滅菌之打孔器切取菌絲邊緣，置於 PSA 平板中央，分別於 15、20、25、30 及 35℃之定溫箱中黑暗培養 4 wk 後測量菌絲生長直徑，每處理 6 重複，本試驗重複 2 次。另外將 RSB-5 與 RSB-9 分離株以 PSA 培養基培養 2–4 wk (25℃)，待其產生厚膜孢子後，以 0.05% Tween-80 配製孢子懸浮液 (5×10^5 conidia mL^{-1})，吸取 50 µL 孢子懸浮液置於 Difco PDA 平板中央，以滅菌過之 L 型玻棒均勻塗抹，分別置於 15、20、25、30、32、34 及 35℃之定溫箱中，黑暗靜置 48 h 後，取出培養皿於顯微鏡下觀察厚膜孢子之發芽情形，發芽菌絲長度達厚膜孢子寬度 2 倍則視為發芽，於顯微鏡下計算 100 個孢子的發芽率，每處理 4 重複，本試驗重複進行 2 次。

化學藥劑對稻麴病菌菌絲生長及孢子發芽之影響

將 PSA 培養之 RSB-5 與 RSB-9 菌株厚膜孢子以 0.05% Tween-80 配製成孢子懸浮液 (1×10^6 conidia mL^{-1})，吸取 100 µL 上述孢子懸浮液以滅菌過之 L 型玻棒均勻塗抹於 PSA 平板上，置於 25℃定溫箱培養 1 wk 後，以直徑 0.5 cm 滅菌之打孔器切取菌絲塊供試，並利用藥劑平板測試法於室內測定供試藥劑之抑菌效果。以目前推薦在水稻真菌性病害防治之藥劑包括：23.6% 百克敏水懸劑 (pyraclostrobin (suspension concentrate; SC)，大成化學工業股份有限公司，臺灣臺中市)、5% 嘉賜黴素溶液 (kasugamycin (solution; SL)，大勝化學工業股份有限公司，臺灣臺北市)、40% 甲基多保淨水懸劑 (thiophanate-methyl (SC)，立農化學股份有限公司，臺灣雲林縣)、45% 嘉賜三賽唑水懸劑 (kasugamycin + tricyclazole (SC)，大勝化學工業股份有限公司，臺灣臺北市)、33% 鋅錳乃浦水懸劑 (mancozeb (SC)，臺灣道禮股份有限公司，臺灣臺北市)、40% 克熱淨可濕性粉劑 (iminoctadine tris (albesilate) (wettable powder; WP)，億豐農化廠股份有限公司，臺灣臺中市)、50% 免賴得可濕性粉劑 (benomyl (WP)，富農化學股份有限公司，臺灣桃園市) 7 種供試藥劑，依推薦倍數配製成含藥之 PSA 培養基，以不加藥劑之 PSA 處理做為對照組。將直徑 0.5 cm 菌絲塊菌絲面朝下置入直徑 8.5 cm 之含藥 PSA 平板中央，置於 25℃定溫箱黑暗培養 4 wk 後，測量其菌絲生長直徑。每處理 6 重複，本試驗重複進行 2 次。試驗結果按下列公式換算藥劑對菌絲之生長抑制率：菌絲生長抑制率 (%) = {[對照組生長直徑 (cm) − 藥劑處理組生長直徑 (cm)]/對照組生長直徑 (cm)} × 100%。

另以 0.05% Tween-80 配製孢子懸浮液 5×10^5 conidia mL^{-1}，分別吸取 50 µL 孢子懸浮液置於不同藥劑 Difco PDA 平板中央，以滅菌過之 L 型玻棒均勻塗抹。置放於 25℃定溫箱中，黑暗靜置 48 h 後，取出於顯微鏡下逢機計算 100 個孢子之發芽率，每處理 4 皿重複，本試驗重複進行 2 次，另以不加藥劑之 PDA 處理做為對照組。試驗結果按下列公式換算藥劑對厚膜孢子發芽抑制率：孢子發芽抑制率 (%) = {[對照組孢子發芽率 (%) − 藥劑處理組生孢子發芽率 (%)]/對照組孢子發芽率 (%)} × 100%。

統計分析

各項處理的試驗資料利用 SAS Enterprise Guide 7.1 版統計分析軟體先進行變方分析 (analysis of variance; ANOVA)，再以最小顯著性差異 (least significant difference; LSD) 測驗，在 5% 顯著水準下比較處理間平均值之差異。統計繪圖則使用 IBM SPSS Statics 25.0 版本繪製。

結果

水稻稻麴病的發生

本試驗於 2022–2023 年調查 2 年共 4 期

作水稻稻麴病發生情形，其中稻麴病僅發生於 2022 年第一期作與 2023 年第二期作，其病徵為罹病穀粒呈現黃褐色菌塊 (圖 1A)，外層薄膜破裂時，裸露黃褐色厚膜孢子 (圖 1B)。田間稻株稻麴病發生率之調查顯示，2022 年第一期作，A、B 兩調查點之稻欉平均穗數分別為 19.9 與 17.8 穗，稻欉罹病率為 30.7 與 27.5%，罹病穗率為 9.6 與 10.6%；2023 年第二期作，A、B 兩調查點之稻欉平均穗數分別為 13.9 與 14.5 穗，稻欉罹病率則分別為 2.8% 與 3.0%，罹病穗率為 8.5 與 7.4% (表 1)。進一步調查稻麴病在病穗之上、中、下段分布位置比率，結果顯示稻麴位於稻穗下段比率最高 (40.9–54.1%)，其次為中段 (36.2–41.2%)，上段之分布比率最少 (7.7–22.7%) (圖 1C 與圖 2)。罹病穗之稻麴數如圖 3 所示，兩次調查結果皆顯示稻麴數以 1 粒 (64.0–75.0%) 與 2 粒 (9.1–21.5%) 所占之比率較高，單穗最高紀錄可達 8 粒，平均每穗稻麴數介於 1.4–2.0 粒。

2019–2023 年兩期作間，水稻生育階段自

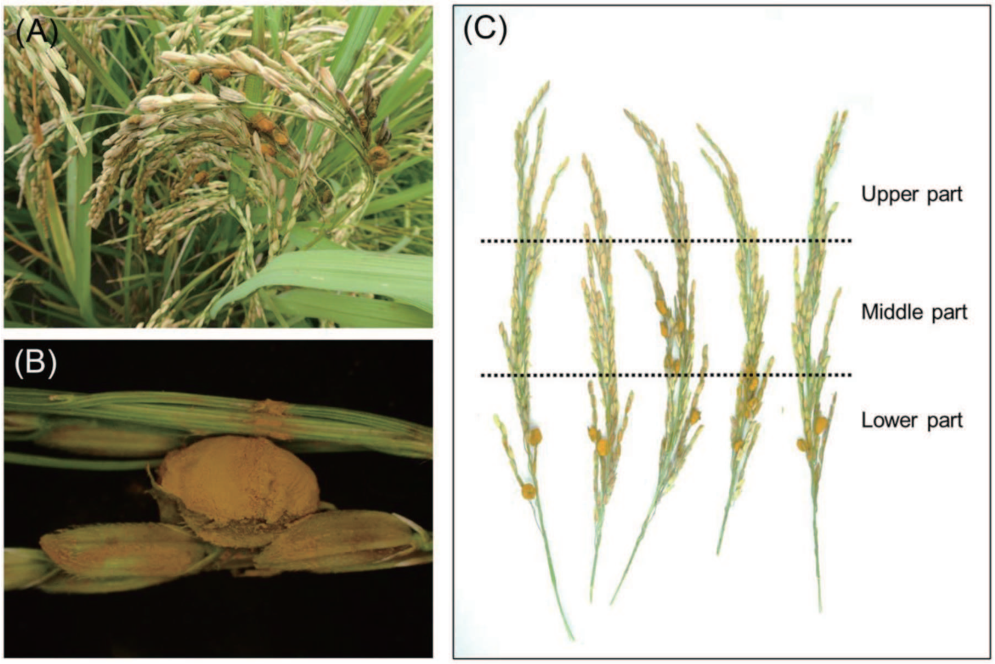

圖 1. 稻麴病之發病情形。(A) 田間罹病穗上形成稻麴；(B) 稻麴表面布滿黃色厚膜孢子；(C) 將稻穗平均分為上、中、下段，並記錄稻麴於病穗上之分布位置。

Fig. 1. The occurrence of rice false smut disease: (A) formation of smut balls on infected panicles in the field; (B) surface of smut ball covered with yellow chlamydospores; and (C) division of the rice panicle into upper, middle, and lower parts to record the distribution of smut balls on the infected panicle.

表 1. 嘉義縣民雄鄉水稻稻麴病之發生情形調查。

Table 1. The occurrence of rice false smut investigated in Minxiong Township, Chiayi County from 2022 to 2023.

Cropping season	Cultivars	Site[z]	No. panicle/hill	Infected hill (%)	Infected panicle (%)
1st crop, 2022	TNS 22	A	19.9 ± 2.8	30.7 ± 5.1	9.6 ± 1.3
		B	17.8 ± 0.2	27.5 ± 7.7	10.6 ± 1.3
2nd crop, 2023	KH 147	A	13.9 ± 0.5	2.8 ± 1.4	8.5 ± 0.3
		B	14.5 ± 0.8	3.0 ± 0.8	7.4 ± 0.8

[z] A: 23°34'02.8"N 120°27'16.2"E; B: 23°34'02.9"N 120°27'15.4"E.

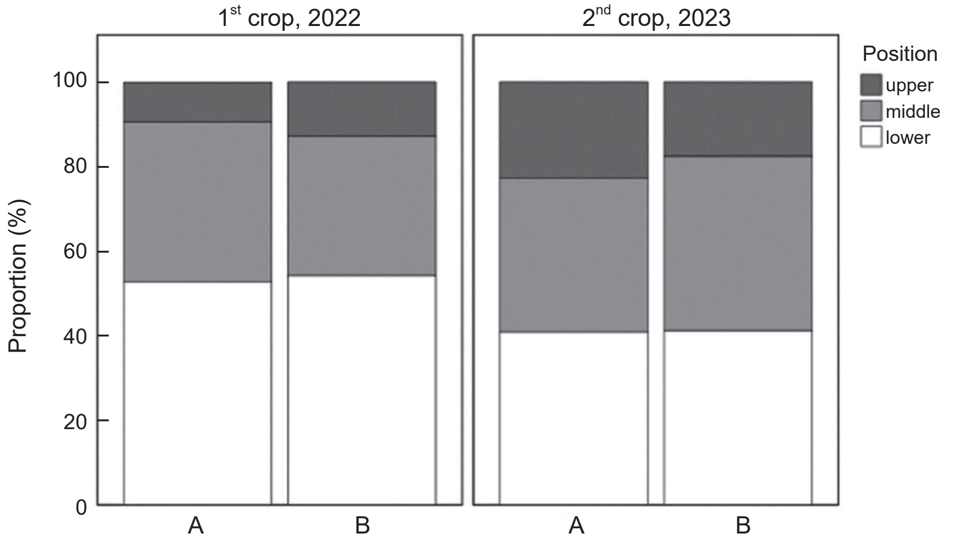

圖 2. 稻麴在罹病穗之上、中、下段之分布情形 (A: 23°34'02.8"N 120°27'16.2"E; B: 23°34'02.9"N 120°27'15.4"E)。

Fig. 2. The distribution of smut balls on the upper, middle, and lower parts of infected panicles.

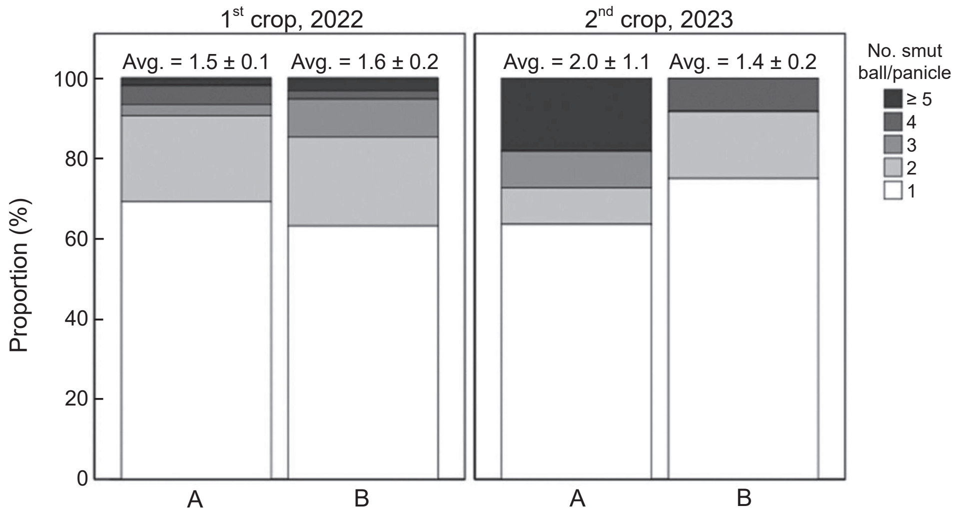

圖 3. 罹病穗上形成不同數量稻麴之比率 (A: 23°34'02.8"N 120°27'16.2"E; B: 23°34'02.9"N 120°27'15.4"E)。

Fig. 3. The proportion of different numbers of smut balls formed on infected panicles.

幼穗形成期至乳熟期之氣象資料如圖 4 所示，圖 4A 顯示不同年份之第 1 期稻作經歷之平均氣溫，其中 2022 年田間稻麴病嚴重發生，抽穗前後各經歷一次 5 年來低溫之極端離群值 (extreme outlier) 分別為 17.4℃與 17.6℃，其他年份則顯示日均溫皆在 20℃以上，該年份一期作抽穗期前後之平均相對濕度 (84.7 與 81.9%) 與其他年份之間無明顯差異 (72.5–83.2% 與 73.0–85.3%) (圖 4B)，但在抽穗前累積降雨量為所有年份中最高 112.5 mm，抽穗後之累積降雨量為 198.5 mm，僅次於 2020 年之 290 mm (圖 4C)；2023 年第二期作田間亦發生稻

麴病，在日均溫方面與其他年份相比差異不大，抽穗前日均溫皆高於 25℃ (26.7–29.1℃)，抽穗後溫度稍有下降，仍介於 24.2–25.6℃ (圖 4D)，但在相對濕度與累積降雨量方面，2023 年與其他年份之間差異明顯，抽穗前平均相對濕度與累積雨量皆明顯高於其他年份，分別為 89.9% 與 37 mm，抽穗後雖未降雨，平均相對濕度仍有 83.6%，相較其他年份於抽穗前後平均相對濕度皆未達 80% (圖 4E、F)。

稻麴病菌的分離與培養

本試驗自供試田區共收集了 9 株稻麴病菌分離株，其菌落型態差異大，利用 PSA 培養基於 25℃下培養 4 wk，其中 RSB-2、RSB-3 及 RSB-6 等 3 株菌株之生長狀態僅有菌絲型態，其他 6 株菌株則在菌落中央產生厚膜孢子，菌落正面呈白色、黃色或黃綠色，部分菌株中央呈深褐色 (圖 5A)，菌落背面呈淺黃色與黃綠色，部分菌株分泌褐色或黑褐色色素 (圖

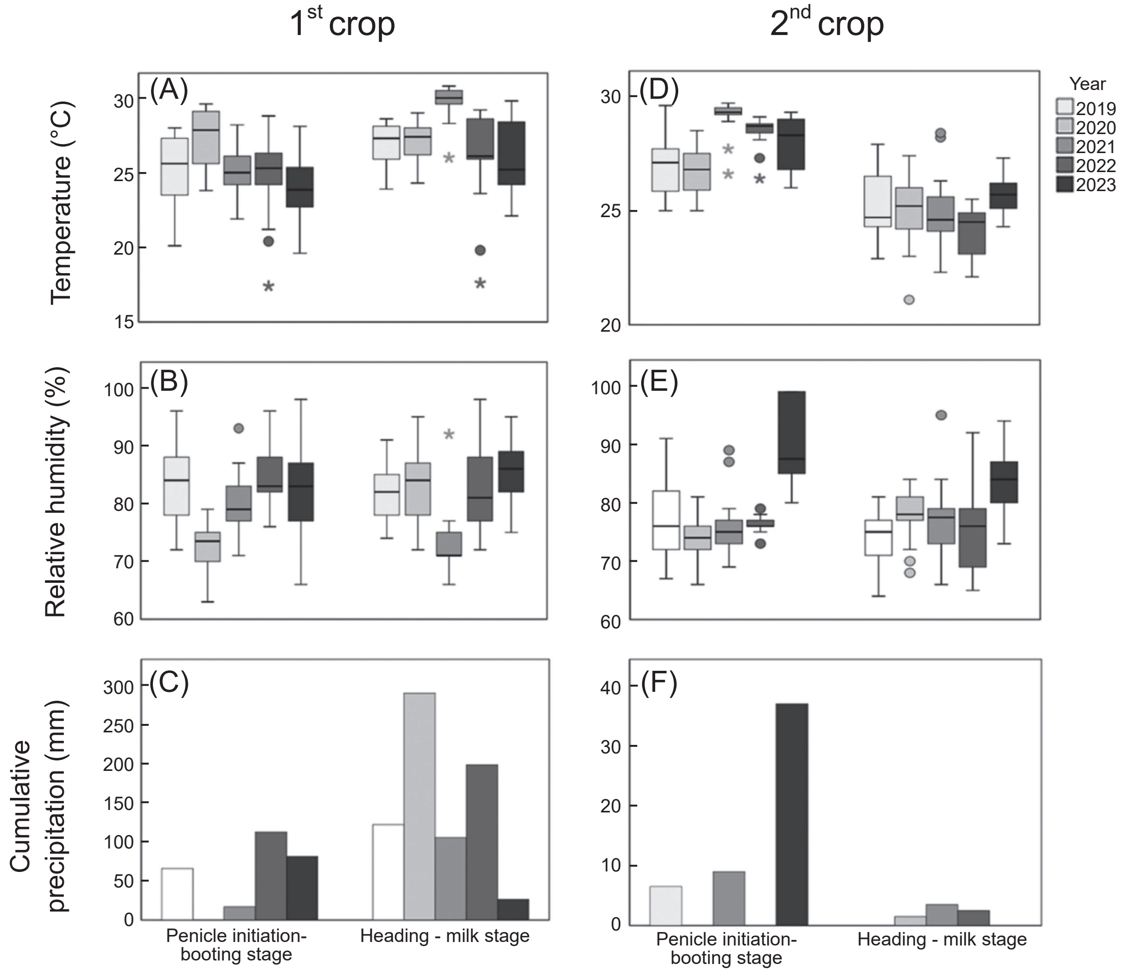

圖 4. 2019–2023 年水稻調查田區不同期作『幼穗形成期-孕穗期』與『抽穗期-乳熟期』生育階段之氣象資料。1 期稻作之 (A) 日均溫 (B) 相對濕度及 (C) 累積降雨量。2 期稻作之 (D) 日均溫 (E) 相對濕度及 (F) 累積降雨量。

Fig. 4. Meteorological data for different rice cropping seasons during the "panicle initiation to booting stage" and "heading to milk stage" in surveyed fields from 2019 to 2023: (A) daily average temperature; (B) relative humidity; and (C) cumulative rainfall for the first cropping season; (D) daily average temperature; (E) relative humidity; and (F) cumulative rainfall for the second cropping season.

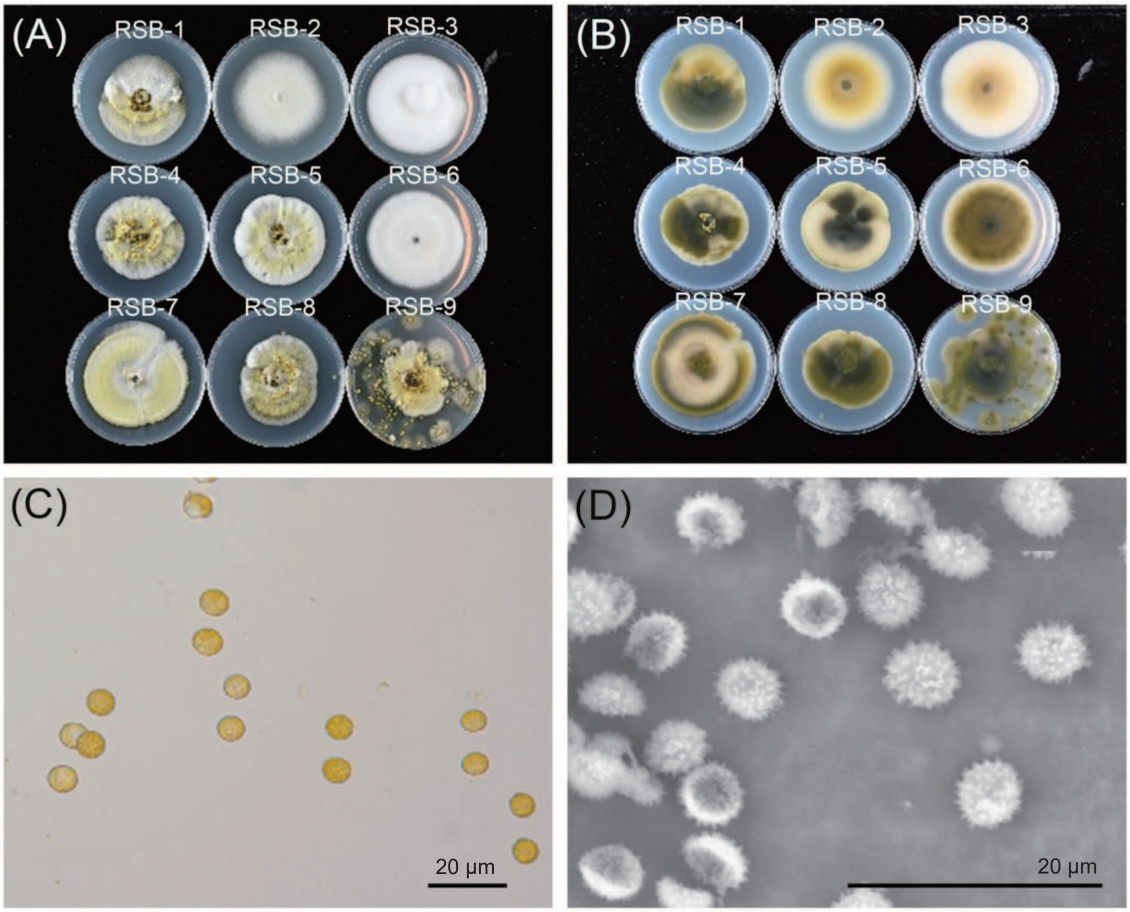

圖 5. 稻麴病菌菌落與厚膜孢子形態。9 株分離株以 PSA 培養 4 wk 之 (A) 正面與 (B) 反面菌落型態。以 (C) 干涉位像差顯微鏡與 (D) 掃描式電子顯微鏡觀察之厚膜孢子形態。

Fig. 5. The morphology of colonies and chlamydospores of *Ustilaginoidea virens*. Nine isolates (RSB-1 to RSB-9) were cultured on potato sucrose agar for 4 wk, and the appearance of colony showed on the (A) front and (B) reverse sides. The morphology of chlamydospores under (C) the differential interference contrast microscopy and (D) the scanning electron microscope.

5B)，取菌株 RSB-5 與 RSB-9 進行觀察，其厚膜孢子黃綠色圓形並於表面有針狀突起物 (圖 5C、D)，另外所測量之 RSB-5 菌株厚膜孢子長寬為 6.49 ± 0.06 µm × 6.12 ± 0.06 µm，RSB-9 之厚膜孢子長寬為 6.99 ± 0.05 µm × 6.50 ± 0.07 µm (表 2)。菌株於 25℃下以 PSA 培養 4 wk，菌落直徑範圍為 4.9–6.9 cm，其中 RSB-9 菌落直徑僅 4.9 cm (表 2)，但厚膜孢子產生速度快。

稻麴病菌分子鑑定與其交配型分析

挑選不同菌落型態之菌株 RSB-1、RSB-2、RSB-3、RSB-5、RSB-6 及 RSB-9 進行 ITS 序列之增幅及定序，發現 6 菌株彼此之序列相似度為 100%，其中 RSB-5 與 RSB-9 之序列已登錄於 NCBI GenBank 資料庫 (Accession no. PP389938 與 PP389939)，續將本序列於 NCBI GenBank 網站進行比對，比對結果與 *U. virens* 菌株 118-CLR-KA-40 的 ITS 序列 (Accession no. OL744511) 有高達 99.69% 之相似度。為進一步瞭解所收集之菌株交配型之種類，由專一性引子對偵測 6 株稻麴病菌之交配型，發現 6 株菌皆可增幅出 MAT1-1 片段，無法增幅出 MAT1-2 (圖 6)，顯示 6 株稻麴病菌為 MAT1-1 交配型。

表 2. 稻麴病菌以 PSA 培養基在 25℃下培養 4 wk 之菌絲生長直徑及厚膜孢子大小。

Table 2. The mycelial growth and chlamydospore size of *Ustilaginoidea virens* on potato sucrose agar (PSA) at 25℃ for 4 wk.

Isolates	Mycelial diameter (cm)	Chlamydospore length/width (μm)
RSB-1	5.7 ± 0.0 cd[z]	-
RSB-2	6.1 ± 0.1 bc	-
RSB-3	6.5 ± 0.2 ab	-
RSB-4	5.3 ± 0.3 de	-
RSB-5	5.6 ± 0.1 d	6.49 ± 0.06/6.12 ± 0.06
RSB-6	6.4 ± 0.1 ab	-
RSB-7	6.9 ± 0.2 a	-
RSB-8	5.0 ± 0.3 e	-
RSB-9	4.9 ± 0.1 e	6.99 ± 0.05/6.50 ± 0.07
LSD	0.5	-

[z] Means in each column with different letters represent significant differences ($P < 0.05$) by least significant difference (LSD) test.

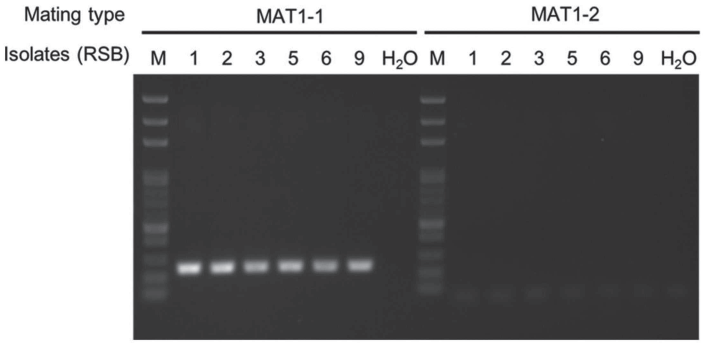

圖 6. 6 個稻麴病菌菌株 (RSB-1、2、3、5、6 及 9) DNA 以不同交配型 (mating type) 專一性引子對 (MAT1-1 與 MAT1-2) PCR 增幅之結果。M: DNA marker。

Fig. 6. The PCR amplification of 6 *Ustilagionoidea virens* isolates (RSB-1, 2, 3, 5, 6, 9) genomic DNA with mating type specific primers (MAT1-1, MAT1-2). M: DNA marker.

溫度對稻麴病菌菌絲生長與孢子發芽之影響

由溫度對 RSB-5 與 RSB-9 菌株菌絲生長影響試驗，顯示兩菌株皆於 25℃菌絲生長最佳，分別為 7.1 與 6.3 cm，20℃次之，分別為 5.0 與 4.8 cm，在 30℃培養下，菌絲直徑為 3.5 與 4.5 cm，而 35℃下菌絲完全不生長 (表 3)。另外，進行供試溫度對 RSB-5 與 RSB-9 菌株孢子發芽影響試驗 (表 4)，結果顯示 2 菌株在 25℃時有較高的發芽率，分別為 79.3% 與 77.8%，於 20℃與 30℃培養時，發芽率在 58.5–74.5%，隨溫度增加而遞減，35℃時完全不發芽。

化學藥劑對稻麴病菌菌絲與孢子生長之影響

於室內測試百克敏、嘉賜黴素、甲基多保淨、嘉賜三賽唑、鋅錳乃浦、克熱淨及免賴得等 7 種殺菌劑對 RSB-5 與 RSB-9 菌株菌絲生長之影響 (表 5)，結果顯示百克敏、鋅錳乃浦及克熱淨對 RSB-5 菌株之菌絲生長抑制率可達 100%，免賴得對菌絲生長抑制效果亦可達 95.8%，嘉賜三賽唑、甲基多保淨及嘉賜黴素對菌絲生長抑制率分別為 59.9、19.2 及 3.1%。供試藥劑對 RSB-9 菌株菌絲生長抑制效果，大致與上述 RSB-5 菌株相似，百克敏、鋅錳乃浦、克

表 3. 稻麴病菌 RSB-5RSB-9 菌株在不同溫度下培養 4 wk 之菌絲生長情形。

Table 3. The mycelial growth of *Ustilaginoidea virens* isolates RSB-5 and RSB-9 cultured at different temperatures for 4 wk.

	Colony diameter (cm)	
Temperature (℃)	RSB-5	RSB-9
15	1.9 ± 0.11 d[z]	2.3 ± 0.04 d
20	5.0 ± 0.08 b	4.8 ± 0.05 b
25	7.1 ± 0.04 a	6.3 ± 0.04 a
30	3.5 ± 0.27 c	4.5 ± 0.21 c
35	0.0 ± 0.0 e	0.0 ± 0.0 e
LSD	0.4	0.3

[z] Means in each column with different letters represent significant differences ($P < 0.05$) by least significant difference (LSD) test.

表 4. 不同溫度對稻麴病菌 RSB-5 與 RSB-9 菌株厚膜孢子發芽之影響。

Table 4. Effect of temperatures on the germination rate of chlamydospores of *Ustilaginoidea virens* isolates RSB-5 and RSB-9.

	Germination (%)	
Temperature (℃)	RSB-5[z]	RSB-9
15	53.0 ± 0.4 d	30.5 ± 1.9 e
20	70.0 ± 0.7 c	64.3 ± 1.7 b
25	79.3 ± 1.6 a	77.8 ± 1.7 a
30	74.5 ± 1.8 b	58.5 ± 1.7 c
32	24.0 ± 1.5 e	47.3 ± 1.4 d
34	1.5 ± 0.7 f	1.5 ± 0.3 f
35	0.0 ± 0.0 f	0.0 ± 0.0 f
LSD	3.3	4.1

[z] Means in each column with different letters represent significant differences ($P < 0.05$) by least significant difference (LSD) test.

熱淨及免賴得皆可達 100% 之菌絲抑制率，其次為嘉賜三賽唑對菌絲生長有 63.5% 的生長抑制率，而甲基多保淨與嘉賜黴素則分別僅有 12.0 與 0.2% 之菌絲生長抑制率。

進行上述供試藥劑對RSB-5與RSB-9菌株厚膜孢子發芽影響之試驗 (表 6)，結果顯示厚膜胞子以百克敏、甲基多保淨、鋅錳乃浦、克熱淨及免賴得處理後，對其孢子發芽抑制率皆可達 100.0%，嘉賜三賽唑對 RSB-5 與 RSB-9 厚膜胞子發芽抑制率分別為 83.8 與 78.6%，而嘉賜黴素僅對 RSB-5 與 RSB-9 則有 57.0 與 63.0% 之厚膜孢子發芽抑制率。

討論

稻麴病之發生在過去文獻顯示與水稻栽培期間之物候條件密切相關 (Iijima & Konuma 1983; Alase *et al*. 2021)，以往臺灣紀錄稻麴病好發於第 2 期稻作 (Tsai 2007)。Tsai *et al*. (1990) 曾於 1983 年與 1988 年調查臺灣嘉南地區水稻二期作之稻麴罹病率，結果顯示 1983 年的罹病欉率可達 19.3%、罹病穗率最高為 14.9%，而 1988 年的罹病穗率最高為 12.5%。本研究於 2023 年第 2 期稻作調查稻麴病罹病欉率最高僅為 3.0%，反而在 2022 年第一期作觀察到高達 30.7% 的罹病欉率與 10.6% 的罹病穗率，推測可能與降雨量及抽穗前氣溫異常有關。過去許多調查報告水稻抽穗期前後如遭遇連續降雨及高相對濕度 (> 80%) 易造成稻麴病之發生 (Suzuki 1981; Iijima & Konuma 1983; Fan *et al*. 2014; Tanaka *et al*. 2017; Alase *et al*. 2021)。溫度是加劇稻麴病菌危害之另一重要因子，本研究於 2022 年之調查發現，嘉義地區第 1 期稻作罹病欉率高達 27.5–30.7%，且該年度桃園、新竹、苗栗及花蓮地區 1 期稻作皆普遍發生稻麴病 (Lai & Wang 2022)，由氣象資料分析顯示，2022 年 5 月上旬嘉義地區之水稻生育正值幼穗形成期至孕穗期，整體氣溫較常年低 1.5℃，最低日均溫達 17.4℃，加上雨量及降雨日數偏多 (CWA 2023)，推測為稻麴病嚴重發生之原因。Alase *et al*. (2021) 認為稻麴病之嚴重程度與最低溫度呈高度相關性，稻麴病發生較嚴重的年份也常發現與水稻在孕穗期間曾經歷低溫有關 (Suzuki 1981; Iijima & Konuma 1983; Sasahara *et al*. 2008a; Tanaka *et al*. 2012)，許多研究透過人工接種稻麴病菌也證實，接種後之感染階段經歷 2 d 低溫 (15–16℃)，並在感染後置於高濕與 26℃以上之環境下，顯著提升接種成功率 (Fujita *et al*. 1989; Sonoda *et al*. 1997; Ashizawa *et al*. 2011)，對於稻麴病菌而言，低溫雖然是不利生長之條件，但可能藉由促進真菌毒素 ustilaginoidin 生合成基因 *uvpks1* 表現，而增加產孢量、

表 5. 不同殺菌劑抑制稻麴病菌 RSB-5 與 RSB-9 菌株菌絲生長影響。

Table 5. Effect of fungicides on the inhibition rate of mycelial growth of *Ustilaginoidea virens* isolates RSB-5 and RSB-9.

Fungicides	Inhibition (%)[z]	
	RSB-5	RSB-9
23.6% Pyraclostrobin SC[y]	100.0 ± 0.0 a[x]	100.0 ± 0.0 a
5% Kasugamycin S	3.1 ± 1.1 e	0.2 ± 0.2 d
40% Thiophanate-methyl SC	19.2 ± 1.7 d	12.0 ± 2.1 c
45% Kasugamycin + tricyclazole SC	59.9 ± 0.8 c	63.5 ± 1.8 b
33% Mancozeb SC	100.0 ± 0.0 a	100.0 ± 0.0 a
40% Iminoctadine tris (albesilate) WP	100.0 ± 0.0 a	100.0 ± 0.0 a
50% Benomyl WP	95.8 ± 1.9 b	100.0 ± 0.0 a
LSD	3.1	3.0

[z] Inhibition rate (%) = [(Diameter of mycelial growth on PSA without fungicide – diameter of mycelial growth on potato sucrose agar (PSA) with fungicides)/diameter of mycelial growth on PSA without fungicide] × 100%.
[y] S: solution; SC: suspension concentrate; WP: wettable powder.
[x] Means in each column with different letters represent significant differences ($P < 0.05$) by least significant difference (LSD) test.

表 6. 不同殺菌劑抑制稻麴病菌 RSB-5 與 RSB-9 菌株厚膜孢子發芽之影響。

Table 6. Effect of fungicides on the inhibition rate of chlamydospore germination of *Ustilaginoidea virens* isolates RSB-5 and RSB-9.

Fungicides	Inhibition (%)[z]	
	RSB-5	RSB-9
23.6% Pyraclostrobin SC[y]	100.0 ± 0.0 a[x]	100.0 ± 0.0 a
5% Kasugamycin S	57.0 ± 2.8 c	63.0 ± 2.1 c
40% Thiophanate-methyl SC	100.0 ± 0.0 a	100.0 ± 0.0 a
45% Kasugamycin + tricyclazole SC	83.8 ± 2.1 b	78.6 ± 2.4 b
33% Mancozeb SC	100.0 ± 0.0 a	100.0 ± 0.0 a
40% Iminoctadine tris (albesilate) WP	100.0 ± 0.0 a	100.0 ± 0.0 a
50% Benomyl WP	100.0 ± 0.0 a	100.0 ± 0.0 a
LSD	3.9	3.6

[z] Inhibition rate (%) = [(Number of spores germinated on potato sucrose agar (PSA) without fungicide – number of spores germinated on PSA with fungicides)/number of spores germinated on PSA without fungicide] × 100%.
[y] S: solution; SC: suspension concentrate; WP: wettable powder.
[x] Means in each column with different letters represent significant differences ($P < 0.05$) by least significant difference (LSD) test.

孢子萌發率及抑制寄主植物之防禦表現 (Xue *et al*. 2024)，因此在短暫低溫後恢復適合感染之環境條件下，有助於稻麴病感染率增加。

除了氣候因子外，水稻品種 (系) 對稻麴病之發生可能也具有明顯影響 (Kurauchi *et al*. 2006)，然相關報告極少。Chou (1967) 曾在同一試區比較水稻「台南 3 號」與「台中 65 號」對稻麴病菌的抗感性，結果顯示，「台南 3 號」罹病穗數為「台中 65 號」之 9.6 倍，顯示「台南 3 號」較「台中 65 號」感病，但此 2 種水稻品種已非現行栽培之主流品種，未來可進一步比較目前較普遍之栽培品種對稻麴病之抗感性，以作為有機栽培水稻品種之參考。此外，國外報告顯示，Ashizawa *et al*. (2011) 經由接種稻麴病菌顯示，一般商業用與抗稻熱病水稻品種皆具有抗性，莖葉高產之飼料用水稻較為感病，但在抗病機制方面尚無定論；Ashizawa *et al*. (2012) 更進一步觀察菌絲入侵過程，建

議抗稻麴病之育種，應朝向篩選穎殼頂端間隙較小或封閉之品種 (系)，但仍需注意的是，即使栽培抗病品種 (系)，氣候因子或肥料過量施用仍會使田間發病率增加。

本研究將田間分離之稻麴病菌以 PSA 培養基進行培養，顯示即使採自同一田區之不同菌株，在培養後呈現之菌落型態、生長速度及產孢量差異明顯。目前許多研究亦指出採集自不同地區之稻麴病菌株，經培養後其菌落顏色、菌絲生長速度、產孢量、厚膜孢子顏色、型態及發芽率有相當大的差異 (Tsai *et al*. 1990; Rani & Sharma 2018; Sharanabasav *et al*. 2021; Fu *et al*. 2022)。Fu *et al*. (2022) 使用 PSA 培養之菌落顏色多數呈黃至淡黃色，其次為白色或黃綠色，少數為黑色，然而菌落特徵與菌株來源之地區並無相關性 (Rani & Sharma 2018)，縱使採集自同一地區之稻麴病菌，遺傳多樣性也相當高，並在菌絲生長速度與致病性上有明顯不同 (Tan *et al*. 2022)，由本研究顯示，同一個地區所採集的菌株確實型態與產胞量均有顯著差異，然其與遺傳多樣性或致病性是否相關仍須進一步研究。不同溫度對稻麴病菌菌絲生長與孢子發芽之影響，顯示 25℃最適合菌絲生長與厚膜孢子發芽，35℃以上則完全被抑制，此與過去水稻稻麴病菌之相關生理研究特性相似 (Hashioka *et al*. 1951; Fujita *et al*. 1990; Tsai *et al*. 1990; Baite & Sharma 2015; Kumar *et al*. 2015; Bhargava *et al*. 2018)。

已知稻麴病菌之生活史包含無性與有性世代，有性世代之基因座會影響稻麴病菌產孢、對逆境的反應及其病原性 (Yong *et al*. 2020)。稻麴病菌有性世代之交配型基因座包含 MAT1-1 與 MAT1-2 兩型，在 2 種交配型之菌株皆存在的狀況下，稻麴病菌於交配後產生有性世代之菌核 (sclerotia) (Sun *et al*. 2020)。從本研究所分離的菌株均為 MAT1-1 交配型，且田間調查時觀察到之病原菌病兆皆為無性世代之厚膜孢子堆，而未觀察到菌核，顯示調查區域可能僅存在 MAT1-1 交配型之稻麴病菌。菌核在稻麴病菌生活史中扮演重要角色，可幫助病原菌抵抗逆境，越冬後發芽產生大量子囊孢子而成為初次感染源，且有性世代基因變異較大，可能經由選汰的過程增加病原菌的適應性。本病在中國、印度等地皆為普遍發生的水稻病害，嚴重時罹病穗率可達 70% 以上 (Ladhalakshmi *et al*. 2012; Kumari & Kumar 2015; Qiu *et al*. 2019)，在前述兩個國家裡，田間存在 2 種交配型之稻麴病菌 (Sharanabasav *et al*. 2021; Fu *et al*. 2022)；在亞熱帶區域，秋天低溫時，病害的嚴重度與菌核田間數量皆顯著增加 (Yong *et al*. 2018)。但在臺灣，對於稻麴病菌交配型的研究極少，本研究僅發現 MAT1-1 交配型之菌株，亦未在調查時發現菌核的存在，此與 Tsai *et al*. (1990) 之研究一致，其亦無觀察到田間有水稻稻麴病菌菌核存在，因此後續應持續監測田間稻麴病的發生、收集更多區域的病原菌菌株，以瞭解田間是否有稻麴病菌 MAT1-2 交配型存在？供未來田間病原菌族群變異性研究之參考，據以擬定相關防治策略。

臺灣目前未有登記水稻稻麴病之藥劑，因此參考水稻其他真菌性病害之藥劑進行室內藥效測試，測試結果顯示，除了嘉賜黴素、嘉賜三賽唑及甲基多保淨無法同時對稻麴病菌菌絲生長與厚膜孢子發芽具明顯抑制效果外，其他測試藥劑如百克敏、鋅錳乃浦、克熱淨及免賴得等均可明顯抑制菌絲生長與厚膜胞子發芽，顯示稻麴病菌對藥劑的感受性仍相當高，此結果與過去研究報告顯示銅劑、鋅錳乃浦、亞托敏、貝芬替、三賽唑、四氯異苯睛，以及混合劑亞托待克利、三氟得克利、免得克敏、貝芬錳等藥劑具有抑制稻麴病菌之效果 (Tsai *et al*. 1990; Dodan & Singh 1996; Tsuda *et al*. 2003; Muniraju *et al*. 2017; Banasode & Hosagoudar 2020) 具有一致性，本次田間調查亦觀察到周邊慣行田區未發生稻麴病之情形，可能與現今用藥種類有關，因而限制病害之發生，然此部分仍需進行長時間且多地區的調查方能結論。惟目前已發現稻麴病菌抗藥性菌株之存在，Zhou *et al*. (2017) 自農藥慣行田分離出對普克利具抗藥性之菌株，半最大效應濃度 (half maximal effective concentration; EC_{50}) 及完全抑制濃度分別為敏感菌株之 224.2 與 40 倍以上，且同時對待克利與得克利具交互抗性，但與作用機制為苯醌外部抑制劑 (quinone outside inhibitor; QoI) 類殺菌劑尚無交互抗性，這種

抗藥性似乎與滲透壓升高及 *CYP51* 基因的表達增強有關，該基因在真菌中的麥角甾醇合成中發揮重要作用，可誘導對麥角甾醇合成抑制劑 (ergosterol biosynthesis inhibitor, EBI) 類殺菌劑的抗藥性 (Zhou *et al*. 2017, 2019)，因此用藥管理上需考量藥劑之輪替使用，以避免加速抗藥性菌株之產生。施藥時機也是另一防治重點，Song *et al*. (2021) 使用殺菌劑對稻種消毒，仍無法減少田間稻麴病發生，日本已建議在抽穗前 1–2 wk 施用防治藥劑，若以粒劑施用，則在抽穗前 2–3 wk 施藥防治稻麴病之效果最佳 (Tsuda *et al*. 2003, 2006)；在有機栽培之防治上，建議於抽穗前 10–14 d 施用波爾多液或銅劑類之藥劑進行防治 (Honkura 1989; NARO 2023)。

除了藥劑防治外，稻麴病亦可透過風險評估 (Ashizawa *et al*. 2010)、氣象預測 (NARO 2018) 及清除中間寄主 (Shetty & Shetty 1985, 1987) 等預防方式，以及應用栽培抗病品種 (Ashizawa *et al*. 2011)、耕作防治 (Ahonsi *et al*. 2000; Sasahara *et al*. 2008b)、土壤處理 (Ashizawa *et al*. 2018) 及肥培管理 (Brooks *et al*. 2009) 等田間管理措施進行防治。日本農業食品產業技術綜合研究機構 (National Agriculture and Food Research Organization; NARO) 亦已建立稻麴病防治之標準作業程序 (NARO 2023)，實際應用於水稻稻麴病之防治，在氣候多變之際，應強化農友對稻麴病的認識，監控田間發生狀況，適時進行病害管理，以避免病害大面積發生，造成農民損失。

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The Occurrence and Fungicide Efficacy Screening for the Control of Rice False Smut in Taiwan

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Abstract

Lai, P. Y., C. J. Wu, and H. F. Ni. 2025. The occurrence and fungicide efficacy screening for the control of rice false smut in Taiwan. J. Taiwan Agric. Res. 74(1):63–79.

Rice false smut (*Ustilaginoidea virens*), traditionally a minor disease in Taiwan, has recently become more prevalent due to the increase in organic farming practices and the impact of climate change. This disease not only affects rice yield and quality but also poses a significant threat to food safety due to the mycotoxins. Given the limited reports on the occurrence of this disease in Taiwan, this study was conducted by a comprehensive two-year, four-crop survey of rice false smut incidence in organic rice fields in Minxiong Township, Chiayi County, from 2022 to 2023. The results indicated that rice false smut was present in the first crop of 2022 and the second crop of 2023, with the highest incidence recorded in 2022. The highest hill infection rates ranged from 27.5% to 30.7%, with panicle infection rates between 9.6% and 10.6%. Most infected panicles had 1−2 smut balls, primarily located in the middle to lower parts of the panicle. Nine isolates of *U. virens* were obtained from the field and cultured on potato sucrose agar (PSA), showing variability in sporulation capacity, colony appearance, and mycelial growth rate. Six isolates produced chlamydospores within 2−4 wk, while the other three did not. Genetic sequencing of six isolates revealed identical ITS sequences, 99.69% similar to *U. virens* isolate 118-CLR-KA-40 (Accession no. OL744511), confirming the identification. Mating type analysis indicated all isolates were belonged to the MAT1-1 type. The study also evaluated the effects of temperature on mycelial growth and chlamydospore germination of the two stable isolates, RSB-5 and RSB-9, with optimal growth and germination at 25℃. Based on the fungicide efficacy tests of mycelial gtrowh and chlamydospore germination of the RSB-5 and RSB-9 isolates, pyraclostrobin, mancozeb, iminoctadine tris, and benomyl showed effective mycelial growth inhibition rates for both isolates, while kasugamycin exhibited the lowest inhibition rate. Additionally, pyraclostrobin, thiophanate-methyl, mancozeb, iminoctadine tris, and benomyl achieved 100% inhibition of chlamydospore germination, whereas kasugamycin + tricyclazole and kasugamycin had lower inhibitory effects. These findings suggest that a rotation of pyraclostrobin, thiophanate-methyl, mancozeb, iminoctadine tris, and benomyl during the booting stage of rice could effectively reduce the impact of rice false smut.

Key words: Rice, *Ustilaginoidea virens*, Disease control.

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Short Communication

Collection, Identification, and Culturing of *Phlebopus* sp. from Taiwan

Shiang-Shiuan Yu[1], Cheng-Yu Tsai[1], and Yun-Sheng Lu[2,*]

Abstract

Yu, S. S., C. Y. Tsai, and Y. S. Lu. 2025. Collection, identification, and culturing of *Phlebopus* sp. from Taiwan. J. Taiwan Agric. Res. 74(1):81–85.

Mushrooms are rich in nutrients and popular delicacy. However, due to limitations in cultivation techniques, some mushrooms that are symbiotic with plants still rely on wild harvesting. Boletes are well-known edible wild mushrooms. In 2024, samples similar to bolete mushrooms were collected in Taichung and Yunlin, Taiwan. Both fruiting bodies were medium to large in size with a dark brown pileus surface and the porous structure of hymenium. Upon vertical sectioning of the pileus, the context was yellow sponge-like tissue. Two isolates, designated Ph-1 and Ph-2, were isolated from these samples. To further identify the two isolates, the internal transcribed spacer (ITS) region of rDNA was amplified by polymerase chain reaction (PCR) with the primers ITS1/ITS4 and sequenced. Phylogenetic analysis preliminarily identified both isolates as *Phlebopus* sp. Additionally, to investigate the optimal cultivation conditions of isolates, their colony lengths were measured on three different media including potato dextrose agar (PDA), potato rice bran (PR), and sawdust potato glucose (SPG) medium. The results showed that Ph-1 grew faster than Ph-2 on all three media, and both isolates exhibited the highest growth rate on the SPG medium. It indicated that sawdust effectively promotes the mycelial growth of *Phlebopus* sp. Bolete mushrooms are well-known for their unique flavor and fleshy texture. This study is the first report to identify the *Phlebopus* sp. isolated from Taiwan by combining phenotype and ITS sequence analysis. To establish commercial cultivation methods for bolete mushrooms in Taiwan, future research will focus on cultivating fruiting bodies and evaluating the feasibility of artificial cultivation.

Key words: Edible bolete, *Phlebopus* sp., Mycelium growth medium.

Mushrooms are rich in nutrients and highly favored by consumers. Due to limitations in cultivation techniques, some mushrooms that are symbiotic with plants still have to be obtained through wild harvesting. Among them, boletes are well-known edible wild mushrooms. They can be recognized by boletoid fruiting bodies, olive-brown spore print, ellipsoidal brown basidiospores, and numerous clamps on the hyphae (Putra *et al*. 2024). In 2024, two fruiting bodies with characteristics similar to bolete mushrooms were collected in Taiwan (Fig. 1). The mycelium-isolate Ph-1 (accession number: TARI-Ph1) was collected from Dongshi, Taichung, and the mycelium-isolate Ph-2 (accession number: TARI-Ph2) was from Douliu, Yunlin. Both fruiting bodies were found to be medium to large in size with a dark brown pileus surface. The data on the size of Ph-1 fruiting bodies was not obtained, so only the data for Ph-2 is presented. The height of the entire fruiting body was approximately 114.32 ± 14.21 mm. The length and width of the pileus were 106.43 ±

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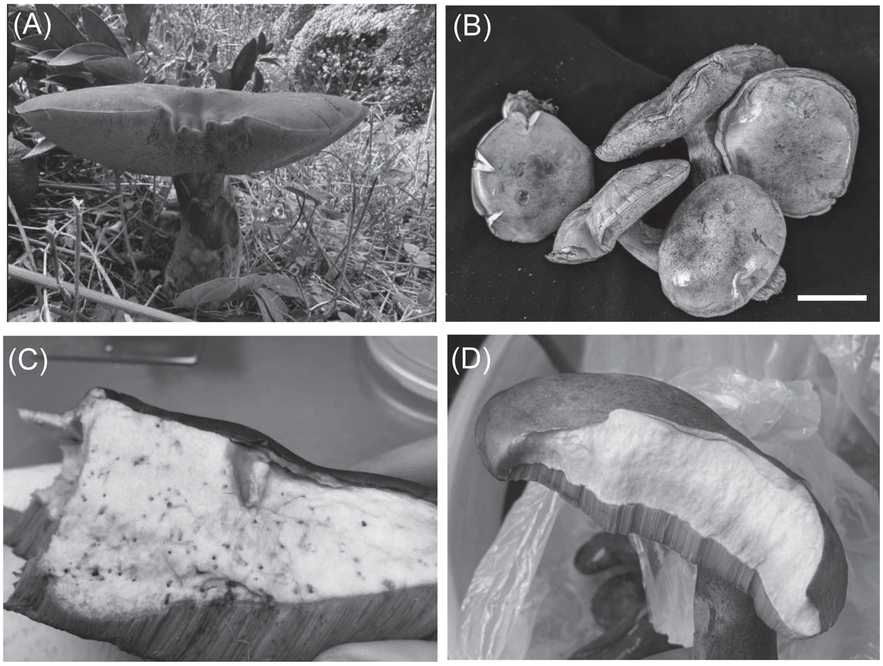

Fig. 1. The fruiting bodies of *Phlebopus* sp. samples. (A) Ph-1 and (B–D) Ph-2. Both fruiting bodies were medium to large in size with a dark brown surface. (C–D) Upon vertical sectioning, light yellow sponge-like tissues were observed. Scale bar = 50 mm. (The size of the Ph-1 fruiting body was not measured.)

9.25, and 86.3 ± 9.49 mm. Upon vertical sectioning of the pileus, light yellow sponge-like tissues were observed. The thickness of the pileus was 21.92 ± 3.28 mm, and the hymenium exhibited a porous structure. The morphology characteristics of the mushroom were similar to *Phlebopus* sp. However, due to the severe decay of the collected fruiting bodies, data on spore prints, basidia, and the number of basidiospores could not be obtained. To further identification, DNA was extracted by Maelstrom Switch 8 Automated DNA/RNA Purification Platform (Taiwan Advanced Nanotech Inc. (TANBead), Taoyuan, Taiwan), and the internal transcribed spacer (ITS) region was amplified by using the universal primers ITS1 and ITS4 (White *et al*. 1990; Blattner 1999). The ITS sequences were BLASTed against the National Center for Biotechnology Information (NCBI) blastn database. The ITS sequences of both isolates had identity with those of *Phlebopus* sp. (Ph-1: query coverage of 100%, identity of 96.62%, e-value of 0.0, NCBI number: MN962562; Ph-2: query coverage of 98%, identity of 85%, e-value of 0.0, NCBI number: MT272131). Six closely related species of Boletinellaceae family were used to construct the phylogenetic tree. ITS Sequences from other fungi were obtained from the NCBI database and aligned using ClustalW multiple alignments (Campi *et al*. 2023; Yang *et al*. 2023; Putra *et al*. 2024). Phylogenetic analysis based on ITS sequences was performed using Mega X (Kumar *et al*. 2018) by Maximum likelihood method (1,000 bootstraps) with Kimura 2-parameter and Gamma Distributed model (K2* + G model) (Fig. 2). Although, the Ph-1 (PQ049143); Ph-2 (PQ061505) can be grouped a complex with the isolates of *P. portentosus* and *P. spongiosus*, but we cannot accurately identify them. Therefore, based on their morphological characteristics, and ITS sequence analysis, the two isolates were preliminarily identified as *Phlebopus* sp. [NCBI number: Ph-1 (PQ049143); Ph-2 (PQ061505)]. Furthermore, the two isolates were cultured in

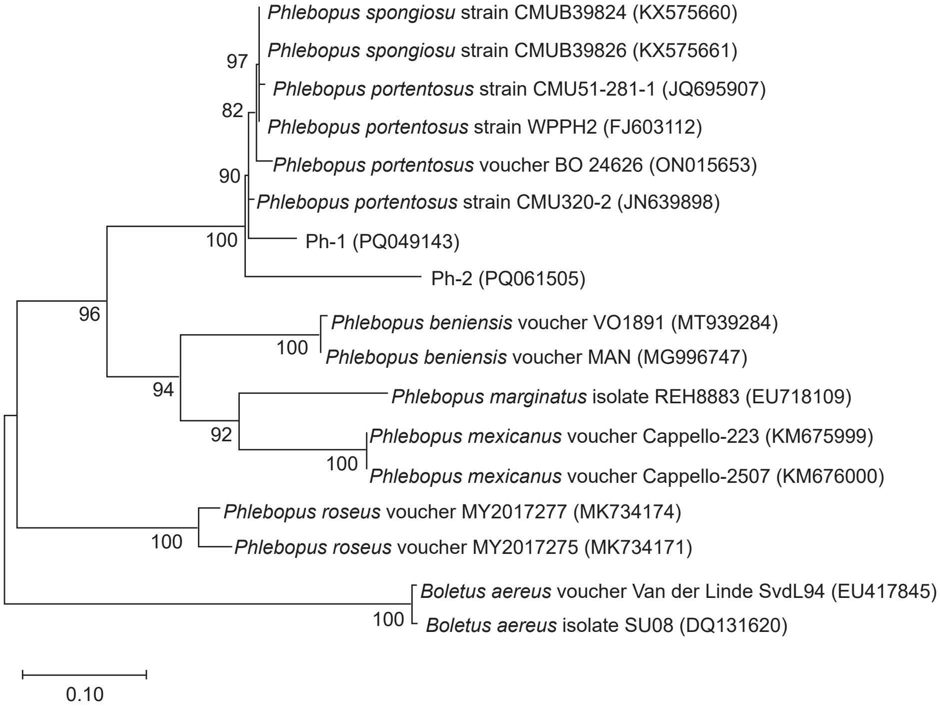

Fig. 2. Phylogenetic tree derived from maximum likelihood method with 1,000 bootstraps based on internal transcribed spacer (ITS) sequences of *Phlebopus* genera using ClustalW multiple alignments on MEGA X. *Boletus aereus* was used as outgroup. Bootstrap values above nodes represent the statistic support for clades. The scale indicates nucleotide substitution per site.

three different media at 28℃ to understand the suitable growth nutrients. The media used in this experiment were as follows: PDA (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), PR (PDA powder 39 g, agar 20 g, rice bran 2 g, K_2HPO_4 1 g, $MgSO_4$ 1 g, $FeSO_4$ 1 g, dH_2O 1 L), and SPG (sawdust 100 g, potato 200 g, glucose 20.85 g, agar 20 g, K_2HPO_4 1 g, $MgSO_4$ 1 g, $FeSO_4$ 1 g, dH_2O 1 L). The experiment was conducted in 2 independent trials, each containing 5 plates per isolate. Data (means ± standard error) with different letters are significantly different according to the least-significant difference test at $P < 0.05$ by using Statistical Analysis System (SAS) version 7.1. The results showed that regardless of the type of culture medium, the mycelium growth rate of Ph-1 was faster than that of Ph-2. Additionally, both isolates had the fastest mycelial growth on SPG after 14 d of cultivation (Table 1). Bolete mushrooms, belonging to the family Boletaceae, are a diverse group of fungi characterized by fleshy fruiting bodies and pores instead of gills on the underside of the cap. For example, *Phlebopus portentosus*, one of Boletaceae family, has been found in tropical and subtropical regions, including China and Northern Thailand. Due to its proteins, amino acids, and minerals, *P. portentosus* is considered a delicious wild edible mushroom (Zhang *et al.* 2017). This study is the first report to identify the *Phlebopus* sp. isolated from Taiwan by combining phenotype and ITS sequence analysis. And the results indicate that *Phlebopus* sp. cannot be identified only by ITS sequence analysis. To further identify the species, additional primers, such as RPB2 and EF1α, will need to be included for multilocus sequence analysis (MLSA) in the future. Moreover, previous studies have also reported that bolete mushrooms have symbiotic relationships with plants, and they can

Table 1. The mycelium growth rate of Ph-1 and Ph-2 on three different media.

	Ph-1[z]			Ph-2		
Day	PDA[y]	PR	SPG	PDA	PR	SPG
7 d	2.25 ± 0.05 a[x]	1.97 ± 0.03 b	1.98 ± 0.04 b	1.25 ± 0.05 d	1.41 ± 0.03 c	1.45 ± 0.07 c
10 d	2.89 ± 0.09 a	2.69 ± 0.07 b	2.82 ± 0.09 ab	2.02 ± 0.05 d	2.20 ± 0.04 c	2.19 ± 0.05 cd
14 d	3.55 ± 0.10 b	3.49 ± 0.05 b	4.26 ± 0.08 a	2.67 ± 0.04 d	2.98 ± 0.04 c	3.10 ± 0.11 c

[z] Ph-1/Ph-2: The colony radius was measured 7, 10, and 14 d after the start of mycelial growth.
[y] PDA: potato dextrose agar medium; PR: potato rice bran medium; SPG: sawdust potato glucose medium.
[x] Data (means ± standard error) with different letters are significantly different according to least significance difference (LSD) test at $P < 0.05$. Colony sizes of two isolates on three media on the same day were combined for analysis.

form ectomycorrhizal structures in the roots. Bolete mushrooms have broad hosts, including *Coffea arabica*, *Citrus grandis*, and *Pinus* sp. (Kumla *et al*. 2012, 2020). The complex symbiotic relationships make the artificial cultivation of boletes challenging. To simulate the natural growth environment of boletes, it is essential to understand the nutrition required for their growth. Kumla *et al*. (2020) found that the optimal growth conditions for the mycelium of *Phlebopus portentous* were 30℃ and pH 5.0. Additionally, they used various solid substrates for mycelium growth. Another study showed that the mixture of rice seed and sorghum grains with sawdust significantly promoted the mycelial growth of *P. portentosus* (Zhang *et al*. 2017). In this study, it was also found that the addition of sawdust in the medium could effectively increase the mycelial growth rate. Therefore, to establish commercial cultivation methods for bolete mushrooms in Taiwan, future research will focus on fruiting body cultivation and evaluating the feasibility of artificial cultivation.

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臺灣脈柄牛肝菌 (*Phlebopus* sp.) 之採集、鑑定與培養

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摘要

余祥萱、蔡承佑、呂昀陞。2025。臺灣脈柄牛肝菌 (*Phlebopus* sp.) 之採集、鑑定與培養。台灣農業研究 74(1):81–85。

菇類營養價值豐富，深受消費者喜愛。由於栽培技術之限制，部分與植物共生之菇類仍仰賴野外採集取得，牛肝菌便是其中著名可食用的野生菇類。2024 年分別於臺灣臺中市東勢區與雲林縣斗六市兩處，採集到外觀疑似牛肝菌之子實體樣本，經組織分離後暫稱為 Ph-1 與 Ph-2。兩樣本之菌傘外觀皆呈暗褐色，背面具有孔狀結構，而菌傘縱剖後，其內部構造呈淺黃色海綿狀組織。為進一步鑑定此兩菌株，本研究對其 rDNA 之內轉錄區間 (internal transcribed spacer; ITS) 進行增幅與序列分析，並由親緣分析結果初步鑑定此兩菌均為脈柄牛肝菌屬 (*Phlebopus* sp.)。此外為開發菌株之最適培養基，以供後續人工栽培參考，利用 3 種不同之營養培養基 (potato dextrose agar (PDA)、potato rice bran (PR) 及 sawdust potato glucose (SPG)) 進行菌絲生長速度測定。結果顯示 Ph-1 之菌絲於所有培養基上生長速度皆較 Ph-2 快，且兩菌株均於 SPG 培養基上具有最佳菌絲生長速度，顯見木屑添加可有效促進脈柄牛肝菌之菌絲生長。牛肝菌以其獨特的風味與肉質口感而聞名，本篇為首次結合子實體表型與 ITS 序列分析鑑定國內脈柄牛肝菌屬之研究，未來將持續進行相關栽培試驗，同時也將評估人工栽培之可行性，以建立臺灣牛肝菌之商業化栽培方法。

關鍵詞：可食用牛肝菌、脈柄牛肝菌屬、菌絲生長培養基。

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